Stock structure and critical habitats for a key apex predator: The broadnose sevengill shark *Notorynchus cepedianus*

by

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Dedication

I dedicate this dissertation to my parents, Richard and Consuelo Roach. Thank you for your unwavering love, support, and understanding. You gave me the strength to believe in myself, and to peruse my dreams. The qualities you have instilled in me have helped me to finish this dissertation and to succeed in life whatever the obstacles. Words cannot express my gratitude and I can only aspire to be such wonderful people and parents as you both are. With eternal thanks…I love you both!

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Declarations and Statements

Declaration of originality

This thesis contains no material that has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis. To the best of my knowledge and belie, this thesis contains no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Ethical Conduct

The research conducted in association with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University. All research was conducted with approval from the University of Tasmania Animal Ethics Committee and the James Cook University Animal Ethics Committee (#A2024) and the Department of Environment and Primary Industry, State Government Victoria (Permit #RP1160).

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Statement of Co-authorship

Chapters 2-4 of this thesis have been prepared as scientific manuscripts as identified on the title page of each chapter. In all cases experimental design, field and laboratory work, data analysis and interpretation, and manuscript preparation were the primary responsibility of the candidate. However, these studies were carried out in collaboration with supervisors and co-authors. Contributions and Institution affiliation of the co-authors are outlined below:

Jayson Semmens *(Institute for Marine and Antarctic Studies)* and Adam Barnett *(James Cook University)* are the primary supervisors for this PhD. They both provided guidance, technical support and advice on field work, analysis and manuscript preparation for this thesis.

Chapter 2

Adam Barnett provided primary support for this chapter. Data for this chapter was provided by Christine C. Bruels *(University of Florida College of Medicine and The Guy Harvey Research Institute)* and Mahmood Shivji *(The Guy Harvey Research Institute)*. Adam Barnett, Christine C. Bruels, Mahmood Shivji and Craig Sherman *(Deakin University)* provided advice on analysis and manuscript preparation. Adam Miller *(Deakin University)* provided assistance with phylogeography analysis and reviewed the manuscript. All other co-authors listed below provided tissue samples and/or reviewed the manuscript. These include; David A. Ebert *(Pacific Shark Research Center, and Department of Ichthyology, California Academy of Sciences, USA; South African Institute for Aquatic Biodiversity, South Africa),* Sebastian Schmidt-Roach *(University of Oldenburg, Germany)*, Charlene da Silva and Christopher G. Wilke *(Resources Research, Department of Agriculture, Forestry and Fisheries, South Africa)*, Craig Thorburn *(Kelly Tarltons Sea Life Aquarium, New Zealand)*, Jeffrey C. Mangel and Joanna Alfaro-Shigueto *(ProDelphinus and Universidad Cientifica del Sur, Lima and the University of Exeter, UK)*, Juan Manuel Ezcurra *(Monterey Bay Aquarium, California USA)*, Alejo Irigoyen *(Centro para el Estudio de Sistemas Marinos (CESIMAR), Consejo Nacional de Investigaciones Científicas y Técnicas (CCT*

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Adam Barnett and Paul Hamer (Victorian Fisheries Authority) provided advice on experimental design, assisted with fieldwork and manuscript preparation. Craig Sherman provided assistance with laboratory work, analysis and advice on manuscript preparation. Adam Miller and Mun Hua Tan provided assistance with bioinformatics analysis and reviewed the manuscript. Alison Kock provided samples from South Africa. Adam Barnett was responsible for sourcing the funding for this study.

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Jayson Semmens, Adam Barnett and Paul Hamer provided advice on experimental design, assisted with fieldwork, and provided advice on manuscript preparation.

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General Abstract

Sharks are ecologically important marine animals and currently among the most threatened. Gaps in knowledge about the ecology and behaviour of many species continue to hinder our ability to effectively conserve and manage these animals. Understanding the population structure and movement patterns can provide valuable information on their stock structure and connectivity, behaviour and identify key habitats. This information can then be used to identify possible threats and devise effective management and conservation strategies.

The broadnose sevengill shark (*Notorynchus cepedianus*) is an ecologically important apex marine predator. It is commonly found in temperate coastal areas worldwide, nevertheless, similar to other shark species there are gaps in knowledge about its ecology and behavior, as is listed as "Data deficient" on the IUCN red list. In Australia, gaps in knowledge persist with respect to stock structure, identification of key habitats such as nursery/pupping areas, and the understanding of early life-stage behavior and movement for this species. This thesis aims to expand the currently available data on this species in South Eastern Australian waters in a more comprehensive, multi-method approach, and identify levels of population structure and connectivity from global to local scales. Using genetic and telemetric analyses this study aims to elucidate stock structure, movement patterns and identify key habitats in order to provide vital information to develop appropriate management strategies.

The majority of research has been focused on regional or local spatial scales, with little information currently available on this species global population structure. To determine if this species is panmictic or an assemblage of distinct subpopulations across its global distribution, chapter 2 assessed the genetic phylogeny using mitochondrial (mtCR) and nuclear (ITS2) markers sequencing a total of 249 individuals from three oceanic regions (six locations) across *N. cepedianus'* global distribution. Moderate levels of genetic diversity compared to other shark species were observed, with low diversity within oceanic regions. Significant levels of genetic divergence were observed among oceanic regions, indicative of little to no mixing between the populations. Overall, three genetically distinct

populations of *N. cepedianus* were identified across its global distribution, disproving any suggestions of global panmixia. My findings emphasise the necessity for further taxonomical review of this species to determine if regional lineages may be categorised as separate species, particularly the Eastern Pacific population.

In chapter 3, the genetic population structure of *N. cepedianus* across south-eastern Australia was investigated using genotyping by sequencing (GBS). This revealed an overall moderate genetic diversity with little genetic structuring across its Australian distribution. This is indicative of high levels of mixing and genetic connectivity within Australia. This strong intraregional connectivity complements those observed in the previous chapter and stresses the need for multi-jurisdictional management of this species within coastal waters of New South Wales, South Australia, Victoria and Tasmania.

At local scales, key habitats such as feeding, nursery/pupping, and mating areas are essential for the health and proliferation of species. By observing and monitoring the spatial and temporal movement as well as behaviour of individuals, key habitats can be identified and their importance to population stability and propagation revealed. *Notorynchus cepedianus* has been shown to exhibit seasonal movement, in to (spring –summer) and out of (autumn – winter) sheltered coastal bays. This has generally been thought to be associated with feeding behaviour but some evidence suggests the use of coastal bays, as nursery areas may also be important. To identify potential nurseries, seasonal movement and habitat use, I used a threefold methodological approach combing genetic, telemetric and tagging tools. Chapter 4 used acoustic telemetry to elucidate *N. cepedianus* neonate movement patterns and behavior in a Victorian coastal bay, as well as to identify key habitats for this species in Australia. Results revealed the presence of neonates (<80 cm TL) within Port Phillip Bay (PPB) during the autumn-winter months. The majority of tagged neonates were not detected in the bay after July 2015 and remained undetected within the bay for the rest of the study period (2 years). Neonate movement pattern revealed a preference for deeper areas of the bay (>15 m). Long distance movement revealed the connectivity between the different coastal waters of south-eastern Australia, with both neonate and other life-stages moving between the State jurisdictions. Though, a pupping area for *N. cepedianus* was identified in PPB, neonates only spent a few months within the coastal bay. Thus, while Port Phillip Bay is important for the first few months of neonate

development, monitoring and management strategies will need to consider anthropogenic pressures both within, and outside the bay where the majority of their early development occurs.

In summation, this thesis is an important contribution to the further understanding of the ecology of *N. cepedianus*, particularly population structure, identifying key habitats and understanding early life-stage behaviour. Additionally, this thesis also demonstrates the importance of multidisciplinary approaches for providing a more comprehensive assessment of ecological process.

Broadnose sevengill shark (*Notorynchus cepedianus*). Copyright 2018 The State of Victoria (Victorian Fisheries Authority), 1996-2018. *(https://vfa.vic.gov.au/recreational-fishing/recreational-fishing-guide/catch-limits-and-closed-seasons/types-of-fish/sharks-skates-and-rays/shark)*

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1 General Introduction

1.1 Shark Ecology

Sharks are crucial for the stability and health of the marine environment (Stevens et al. 2000, Heithaus et al. 2008, Ferretti et al. 2010). However many shark species are among the most threatened on the planet, with an approximate one-quarter listed as "Threatened" on the IUCN Red list (Lucifora et al. 2011, Dulvy et al. 2014). Many sharks are categorised as K-selected species and thus exhibit slow growth and maturity rates, low fecundity and low intrinsic rate of population growth (Compagno et al. 2005, Musick 2005). Such species are more vulnerable to fishing pressure (target and non-target) and habitat degradation pressures (Simpfendorfer & Kyne 2009, Knip et al. 2010, Lynch et al. 2010, Cosandey-Godin & Morgan 2011). Shark fishing has occurred for centuries but over the last few decades demand for shark fins has further compounded this issue. Additionally, overlaps between shark hotspots and fishing areas in certain regions has been shown due to the expansion of fishing regions, which may potentially increase shark susceptibility to fishing pressure (Queiroz et al. 2016). Inaccurate and nonspecific reporting of shark catches is a major obstacle for management agencies to obtain reliable stock assessments (Worm et al. 2013). It is highly likely that fisheries catch reports (targeted and by-catch) for sharks actually only represent a fraction of total shark mortality (Clarke et al. 2006b). As a result management priorities for some shark species have shifted from concern over sustainability to reduction of extinction risk (Musick & Musick 2011). One of the major factors hindering these efforts is the absence of basic biological and ecological knowledge available on many shark species, with 47% of all sharks and rays identified as "Data Deficient" by the IUCN Red list (Simpfendorfer 2014). This has contributed to mismanagement and decline of many shark species (Casey et al. 1978, Bonifil 1994, Walker

1999, Campana & Gibson 2008, McFarlane et al. 2009, Wallace et al. 2009, Ferretti et al. 2010).

1.2 Population Genetics

Understanding population structure, distribution and movement is paramount in ascertaining an overview of stock structure, connectivity and dynamics of a species. Identification of management units (MU) or populations is the foundation for effective management strategies (Dudgeon et al. 2012). There has often been a mismatch between biological studies and fisheries management, the use of genomic data to understand genetic population structure may help bridge the gap (Reiss et al. 2009). Currently, one of the most powerful tools for understanding population structure, connectivity, health and stability is genetic data. There are several advantages for using genomic data which includes, non-lethal sampling, a representation of the population can be ascertained from minimal samples and the data can be analysed in a variety of ways to provide an overview of population structure. Over the past several decades these methods have uncovered a wealth of previously undiscovered diversity and divergence within species, (Bickford et al. 2007, Schmidt-Roach et al. 2014, Martinez-Takeshita et al. 2015). Many globally distributed marine species, once considered to be single panmictic populations, have been shown to comprise of many genetically distinct subpopulations. Phylogeography, which combines genetics and historical geographical information, is used to understand metapopulation (a group of spatially separated populations of the same species) connectivity at ecological and evolutionary time scales, and can provide insights about present spatial distributions of a species (Kumar & Kumar 2018). Phylogeographic partitioning of several globally distributed shark species, i.e. genetic divergences between conspecifics from different oceanic regions, has also been revealed (Duncan et al. 2006, Keeney & Heist 2006, Schultz et al. 2008, Karl et al. 2011, Clarke et al. 2015). For example, clear oceanic boundaries have been shown for many species between and within the Pacific, Atlantic, Indian and Oceania regions,, particularly within the Pacific and

Atlantic, with east and west genetic divisions (Dudgeon et al. 2012). Consequently, some widely distributed species considered to have large population sizes, were actually found to be comprised of smaller spatially isolated populations and thus require their own management efforts. Additionally, genomic data has been used to reveal kinship (Iacchei et al. 2013, Städele & Vigilant 2016), philopatic behaviour (Feldheim et al. 2013, Sandoval Laurrabaquio-A et al. 2019), population size (Do et al. 2014), management units (Palsbøll et al. 2006) and to identify and monitor trade of certain species (Shivji et al. 2002, Clarke et al. 2006a). However, genetic data is incomplete without an ecological component. Thus, for a more comprehensive understanding of population structure, connectivity and the drivers influencing these dynamics, an ecological element is required such as movement behaviour and habitat use within population boundaries.

1.3 Movement and key habitats

Movement patterns and marine barriers influence population structure and connectivity of sharks, which ultimately assists in defining species' boundaries and distribution (Bonfil 1997, Jones 2006, Robinson et al. 2009, Williams et al. 2012). Large predators typically have large territories and/or travel large distances, making them difficult and often expensive to study (Heithaus et al. 2002). For many highly mobile marine species this has resulted in limited/incomplete information on movement, distribution and factors influencing these patterns (Austin et al. 2004). Advances in animal tracking technology such as acoustic and satellite telemetry have significantly contributed to overcoming these challenges and increased the knowledge on large spatial movement for sharks species (Block et al. 1998, Heupel et al. 2006, Bradford et al. 2011, Heupel & Webber 2012, Queiroz et al. 2016, Abecasis et al. 2018). Similarly, shared data bases and collaborative efforts between research groups provided additional support in addressing these challenges (Dwyer et al. 2015, IMOS 2018).

However an understanding of movement alone is normally insufficient to fully comprehend the ecological dynamics of a species and thus synthesising spatial information with other ecological processes such as behaviour (philopatry), habitat selection (feeding, breeding, nursery areas), and intra- and interspecies dynamics (sex, age and predator-prey behaviour) (Sims 2003, Heupel et al. 2007, Jorgensen et al. 2009, Knip et al. 2010, Speed et al. 2010) is important to better understand a species ecology. In particular, identification of key habitats, such as nursery and pupping areas facilitates a better understanding of movement patterns, meta-population structure and habitat use, necessary for effective stock management (Martin et al. 2007).

Sharks lack larval dispersal and thus offspring dispersal is initially dependant on parental movement capabilities, and the juveniles thereafter. Many species of sharks use coastal bays and areas as breeding nurseries and/or pupping grounds, (Sims et al. 2000, Heithaus 2007, Heupel et al. 2007, Tavares et al. 2016). Heupel et al. (2007) defines a shark nursery as an area where young sharks are abundant, reside and return over a period of time. The fundamental assumptions of a nursery area are that it, provides shelter from predation, an abundance of prey, and an increased survival rate, thus supporting a greater contribution to the adult population (Heupel et al. 2007). However, these assumption may not always be applicable to all nursery areas (Heupel et al. 2018). Studies have indicated prey limitations within shark nursery areas for juvenile scalloped hammerhead shark (Bush & Holland 2002) and blacktip sharks (Heupel & Hueter 2002), suggesting that prey abundance may not be a key driver for these species using certain nursery areas. Additionally, juvenile mortality rates within nursery areas vary substantially between locations and species, suggesting that some areas may not necessarily provide a lower risk to predation (Heupel et al. 2018). Basic knowledge about the general physical features of nursery areas, such as water temperature, salinity and depth is important, as this may affect movement and site fidelity within these areas (Drymon et al. 2014). Thus, an understanding of juvenile behaviour (feeding and social), movement and habitat use within nursery areas is required to determine the benefits of these areas for a particular shark species.

Generally, juvenile shark movement is poorly understood, however some studies have shown

highly mobile behaviour (spatially, diurnally and vertically through the water column), diverse habitat use, and seasonal and site fidelity behaviour (Holland et al. 1992, Castro 1993, Morrissey & Gruber 1993, Merson & Pratt 2001, Rechisky & Wetherbee 2003, Heupel et al. 2004, Hussey et al. 2009, Heupel et al. 2010, McAllister et al. 2015). However, nursery area studies have been focused mainly on tropical shark species (Heupel et al. 2018), whereas some of the most productive and diverse ecosystems are located in temperate regions (Suchanek 1994). Temperate coastal marine ecosystems often have different physical features and higher water temperature variability than their tropical counterparts. This may have a significant influence on the way temperate shark species use and benefit from nursery areas in these regions. Thus, research within temperate habitats and on pelagic, deep-water and temperate sharks is required to better understand the benefits and functions of nursery areas.

Identification and protection of nursery and pupping habitats have been an important part of managing shark stocks (McAllister et al. 2015, Oh et al. 2017, McAllister et al. 2018). The increased protection of early life stages within a nursery can further enhance population recovery and growth (Brewster-Geisz & Miller 2000). Nursery areas may be particularly beneficial to shark species that produce many small young with high mortality rates. Coastal habitats and environmental conditions may provide slight improvements to survival rates, which can have huge benefits through increased recruitment (Heupel et al 2018). However, for shark species that produce fewer but advanced young, the size of the adult stock rather than environmental conditions and survival rates within nursery areas may have a larger influence on recruitment variability. However, considering that many sharks select certain environments to give birth to their young, there must be some selective advantage, such as increase survival rate, where discrete pupping areas occur. Though ensuring juvenile survival is important, the significance of breeding and pre-breeding stocks is equally, if not more important in some scenarios for maintaining a healthy stock population, as depletions in adult populations have direct impacts on reproductive potential and recruitment capacity (Kinney & Simpfendorfer 2009, Heupel et al. 2018). Additionally, studies have shown that the survival of pre-breeding stock greatly influences population maintenance and stability (Cortés 1999, Heppell et al. 1999, Musick 1999, Simpfendorfer 1999, Gallucci et al. 2006). Thus, identifying nursery/pupping

areas is important for understanding population structure and dynamics, for the effective management of shark species.

1.4 Broadnose sevengill shark (*Notorynchus cepedianus*)

The Broadnose sevengill shark (*Notorynchus cepedianus* Peron, 1807, referred to as *N. cepedianus* hereafter) are related to ancient sharks (from the Jurassic Period) and belong to the family Hexanchidae, commonly known as 'Cow sharks' (Compagno 1981). *N. cepedianus* are easily distinguishable from other shark species by their seven pairs of gill slits, compared to five pairs of gill slits for the majority of other species (Last & Stevens 2009). This species is globally distributed across coastal temperate zones, excluding the North Atlantic and is a large apex-predator, reaching lengths up to 3m (Barnett et al. 2012). These sharks are considered to be one of the most abundant predators in shallow coastal areas, especially during the summer months (Ebert 1989, Lucifora et al. 2005, Last & Stevens 2009, Barnett et al. 2010c, Barnett et al. 2011, Dudgeon et al. 2015, Barnett et al. 2017). *N. cepedianus* were previously targeted during the mid-1900's but are currently considered a low monetary value species, mainly caught as a by-catch species (Ebert 2001, Barnett et al. 2012). Additionally, as this species is not directly targeted, recordings of landings (by-catch, mortality or otherwise) are limited and the impact of fisheries pressure on this species is unclear (De Wysiecki et al. 2018). Major gaps in understanding the ecology and stock structure of this species still persist, listed as 'Data deficient' by the IUCN red list. In particular, there is limited information available with respect to population structure, genetic diversity, juvenile occurrence/habitat use and the identification of key habitats. Formerly, *N. cepedianus* were classified as several different species, generally according to their geographical location, before being synonymised into their current *N. cepedianus* denomination (Compagno 1984). Currently, no studies have been conducted on the global population structure of this species. The only existing genetic studies on this species are recent and revealed low genetic diversity and some mixing of animals between regional coastal bays, ~ 1000 km apart along the west coast of the USA (Larson et al. 2015, Larson et al. 2017). Movement studies corroborate these patterns, with individuals travelling between

coastal bays (> 1800 km) along the west coast of USA (California) (Williams et al. 2012). Apart from the Pacific coast of the USA there is limited information about the stock structure and connectivity of this species on both global and regional scales. Catch assessments in the south-west Atlantic (Argentina), indicated that the main threats to *N. cepedianus* stocks in that region were by-catch from trawl and gillnet fisheries and targeted recreational fisheries. It is unknown if similar threats are relevant to sevengill populations in Australia, however this species is not targeted and is considered of low commercial value in this region. The initial step toward effective management is identifying the population structure and possible threats to these populations that may require managing. Additionally, with marine coastal areas increasingly under threat from anthropogenic factors such as; habitat degradation/loss, pollution, climate change and overexploitation, it is essential that key habitats fundamental to the maintenance of healthy stocks, be identified, managed and conserved.

N. cepedianus exhibit ovoviviparous, reproduction and give birth to live young, up to 82 pups in a litter, bi-annually (Ebert 1989, Ebert 1996, Awruch et al. 2014). Based on the presence of neonates and juveniles, nursery areas have been suggested for this species in bays along California and Argentina coastlines, with pupping estimated to occur during spring months (Ebert 1996, Lucifora et al. 2005). However, studies were not conducted to determine residency and site fidelity behaviour. Though this species is commonly found within coastal areas around Tasmania, South Australia, Victoria and New South Wales in Australia, studies on this species have mostly been conducted in Tasmania, with no neonates and few juveniles being observed (Barnett et al. 2010a, Barnett et al. 2010b, Barnett et al. 2010c, Barnett et al. 2011, Barnett et al. 2012, Stehfest et al. 2014). In south-eastern Tasmania, seasonal occurrences of *N. cepedianus* within coastal areas reach peak abundance during the summer months, which has been associated with feeding behaviour (Barnett et al. 2010a, Barnett et al. 2010c, Barnett et al. 2012). Additional research on this species within Australia has been very limited, with some mention as a by-catch species in fisheries reports (Walker et al. 2005, Zhou et al. 2007, 2009) and adult movement and feeding studies (Braccini 2008, Stehfest et al. 2014). None of the above-mentioned studies focused on early life-stages of *N. cepedianus* or reported the abundance of neonates or juveniles in these areas. Thus, at present no nursery or pupping areas

have been identified for this species in Australia. Anecdotal reports exist from fishers of neonate sevengill presence within Port Phillip Bay, Victoria and along the coast of South Australia, but scientific studies are lacking. Further research is required on this species to fill the gaps in ecological knowledge, particularly with respect to population structure, key habitats and juvenile behaviour.

1.5 Thesis objectives and structure

1.5.1 Aims and thesis structure

The overall aims of this thesis were to understand the global population structure of *Notorynchus cepedianus,* with a particular interest in its Australian population. In addition, this study aimed to identify key habitat, nursery/pupping area, for this species in south-eastern Australia. To achieve these objectives a multi-method approach was employed, which are outlined in the corresponding three data chapters of this thesis.

1.5.2 Thesis Structure

This thesis is written and presented in the form of individual paper format, represented by chapters 2 – 4.

In **Chapter 2**, tissues samples collected from *N. cepedianus* across three oceanic regions were processed and mitochondrial DNA (mtDNA) and ITS2 region genetic analysis were used to determine the genetic structure and phylogeography of *N. cepedianus* across its global distribution. This revealed low genetic diversity within oceanic regions and clear divergences between oceanic populations, indicative of divergent clades within this species.

Chapter 3, investigated the genetic population structure and diversity of *N. cepedianus*, across south-eastern Australia. Tissue samples from Tasmania, South Australia, and Victoria were

analysed using the latest Genotyping by Sequencing (GBS) approach. Single nucleotide polymorphisms (SNPs) analysis revealed high levels of connectivity and mixing between the areas, indicative of one panmictic population within Australia. As a result, management of this species should incorporate all states within south-eastern Australia.

In **Chapter 4**, acoustic telemetry was used to understand neonate and juvenile movement of *N. cepedianus*, in order to identify a key habitat for this species in south-eastern Australia. A receiver network established within Port Phillip Bay, Victoria was utilised to monitor seasonality, residency and site fidelity behaviour of acoustically tagged neonate *N. cepedianus*. The Integrated Marine Observing System (IMOS), Animal tracking facility receiver networks were used to monitor long distance movement across south-eastern Australia.

This thesis reiterates that multifaceted research is required to fully comprehend the complexities of meta-populations, stock structure and the interconnected elements within.

2 Global population structure and phylogeography of an ancient shark species, the broadnose sevengill shark, *Notorynchus cepedianus*

2.1 Abstract

Shark movement, population connectivity and distribution are influenced by biogeographic and phylogeographic barriers. Understanding these effects and the resulting population structure and dynamics is imperative for effective management and conservation of shark species. The broadnose sevengill shark, *Notorynchus cepedianus*, is a widely distributed, temperate and coastally associated shark species. To evaluate the genetic structure, diversity and phylogeography of *N. cepedianus*, across their global distribution, we assessed 249 samples from three oceanic regions, South Atlantic, Oceania and Eastern Pacific. Analysis of the mitochondrial control (mtCR) and nuclear ITS2 regions indicated moderate levels of global genetic diversity (haplotype diversity (h) = 0.709 and nucleotide diversity (π) = 0.00591). However, low genetic diversity within oceanic regions was observed, South Atlantic (h = 0.1496 and π = 0.000189), Oceania (h = 0.2357 and π = 0.000295) and Eastern Pacific (h and π = 0). Significant genetic differentiation among all oceanic regions was observed (global φ_{ST} = 0.9789, $P < 0.001$), while no significant difference within regions was evident (mean φ_{ST} = -0.0070, $P > 0.05$), in particular for the Eastern Pacific region where populations were genetically indistinguishable. Time calibrated Bayesian Inference phylogenetic reconstructions indicated that monophyletic regional clades diverged from a common ancestor approximately 0.55 Mya, with the Atlantic and Oceania clades sharing a more recent common ancestor approximately 0.28 Mya before the present day. The timing of these events coincides with the mid to late Pleistocene suggesting that global glaciation cycles have possibly contributed to the

isolation and subsequent vicariant divergence of regional populations. Overall, our data indicates strong regional division and intraregional panmixia.

2.2 Introduction

In the marine environment biogeographic and phylogeographic barriers such as water temperature, upwelling, currents, sea level fluctuations, physical barriers, and resource availability influence animal movement and gene flow, and contribute to observed biogeographic structuring across the world's oceans (Palumbi 1994). The degree of animal movement between habitats (regional and local) determines metapopulation structure and genetic connectivity, and can facilitate or inhibit interactions across geographic subpopulations (Harrison & Hastings 1996, Carroll et al. 2015). Understanding these stochastic biogeographic patterns is important for managing species, particularly for widely distributed and highly-mobile animals, as it can assist in identifying demographically independent populations (Management Units, MUs) (Palsbøll et al. 2006), and help determine population stability through source and sink population dynamics (Gaggiotti 1996).

Advances in genetic analyses have been paramount in elucidating and understanding historical demographic histories and contemporary patterns of population structure. By assessing levels of genetic diversity, connectivity and divergence within and among geographic populations, population structure and abundance of several widely distributed species over temporal and geographic scales have been revealed, e.g. marine mammals (Baker et al. 1994, Carroll et al. 2015, Lah et al. 2016, Thompson et al. 2016); ridley sea turtle, *Lepidochelys* spp. (Bowen et al. 1998); fish (Larson et al. 2014, Rubio-Castro et al. 2016). Many of these datasets have served as effective frameworks for guiding conservation efforts of threatened, invasive, and commercially important species, and enhanced our appreciation of the evolutionary processes that have shaped and continue to shape marine biodiversity across the globe (Bernard et al. 2016, Larson et al. 2017).

2.2 Introduction

Sharks are some of the most widely distributed and ecologically important marine organisms (Camargo-Gamboa et al. 2010). As apex-predators they regulate their prey and mesopredator populations maintaining balance within the ecosystem (Wallach et al. 2015). However, sharks are also one of the most poorly understood and currently threatened group of animals worldwide with an estimated 1 in 4 shark species listed as threatened, and many others listed as data deficient (IUCN 2012, Dulvy et al. 2014). For most species important aspects regarding their abundance, population structure, genetic diversity and evolutionary history remain largely unknown. Genetic studies to date suggest that phylogeographic structuring in sharks is primarily influenced by adult vagility and habitat use (Schultz et al. 2008, Giles et al. 2016). Biogeographic barriers and behaviour (such as philopatry and habitat preference) can inhibit free movement and habitat utilization (Palumbi 1994, Harrison & Hastings 1996, Dudgeon et al. 2012, Carroll et al. 2015), restricting gene flow across geographic ranges and leading to vicariant diversification (Moura et al. 2013). Sharks are some of the most widely distributed and ecologically important marine organisms (Camargo-Gamboa et al. 2010). As apex-predators they regulate their prey and mesopredator populations maintaining balance within the ecosystem (Wallach et al. 2015). However, sharks are also one of the most poorly understood and currently threatened group of animals worldwide with an estimated 1 in 4 shark species listed as threatened, and many others listed as data deficient (IUCN 2012, Dulvy et al. 2014). For most species, important aspects regarding their abundance, population structure, genetic diversity and evolutionary history remain largely unknown. Genetic studies to date suggest that phylogeographic structuring in sharks is primarily influenced by adult vagility and habitat use (Schultz et al. 2008, Giles et al. 2016). Biogeographic barriers and behaviour (such as philopatry and habitat preference) can inhibit free movement and habitat utilization (Palumbi 1994, Harrison & Hastings 1996, Dudgeon et al. 2012, Carroll et al. 2015), restricting gene flow across geographic ranges and leading to vicariant diversification (Moura et al. 2013). Population genetic studies for several shark species have unveiled patterns of restricted gene flow and genetic structuring both between and within ocean basins (reviewed in (Dudgeon et al. 2012)). Specifically, several widely distributed species show patterns of genetic differentiation between ocean basins, e.g. between the Atlantic and Indo-Pacific oceans (scalloped hammerhead, (Duncan et al. 2006); blacktip shark, (Keeney & Heist 2006); silky

shark, (Clarke et al. 2015); tiger shark, (Bernard et al. 2016)), between the northern and southern hemispheres (great white shark, (O'Leary et al. 2015)) and eastern and western Atlantic ocean (species, (Camargo et al. 2016)). Trends suggest that sharks with a preference for coastal habitats show population structuring on a much smaller geographic scale (Keeney et al. 2005, Keeney & Heist 2006, Schultz et al. 2008, Karl et al. 2011, Geraghty et al. 2014) compared to pelagic species which tend to have higher connectivity across oceanic regions (Heist et al. 1996, Castro et al. 2007, Schmidt et al. 2009, Veríssimo et al. 2017). However, it is difficult to predict shark population structure solely based on habitat type as even closely related species exhibit a broad spectrum of life histories, habitat use and movement patterns. Further population genetic and phlyogeographic research on sharks is required to increase our understanding of patterns of population connectivity and dispersal histories across taxonomic groups, and the processes responsible for shaping patterns of genetic diversity observed today.

The hexanchoid sharks (suborder Hexanchoidei) are a highly distinctive group and one of the earliest known lineages of modern sharks, with representatives being found in the fossil record dating back as to the Lower Jurassic (~190 mya) (Rus Hoelzel et al. 2006, Maisey 2012). The six- and sevengill sharks (family Hexanchidae) are considered primitive among the modern sharks, with distinguishable features including six or seven paired gill openings, a single dorsal fin, and an anal fin (Ebert et al. 2013). Current taxonomy recognises three genera within the Hexanchidae, two of which are monotypic, and collectively have five living species (Naylor et al. 2012, Ebert et al. 2013). The genus *Notorynchus* Ayres, 1855 is considered to be monotypic and consists of the wide-ranging temperate water broadnose sevengill shark, *Notorynchus cepedianus* (Péron 1807b). However the species taxonomy remains contentious, with various justifications for the recognition of distinct species at regional scales (Péron 1807a) and 12 nominal names including *Heptranchias pectorosus* Garman 1884 (Argentina), *Notidanus ferox* Perez Canto 1886 (Chile), *Heptranchias haswelli* Ogilby 1897 (South Africa), *Notidanus medina*e Philippi 1902 (Chile), *Notidanus wolniczkyi* Philippi 1902 (Chile), *Heptranchias spilotus* Lahille 1913 (Argentina), *Notorynchus ocellatus* Devincenzi 1920 (Uruguay), and *Notorynchus macdonaldi* Whitley 1931 (Australia).

2.2 Introduction

The sevengill shark is a coastally-associated circumglobal species that is distributed throughout temperate waters (except for the North Atlantic Ocean), common to inshore bays and estuaries, and depths exceeding 300 m on the continental shelves (Last & Stevens 2009, Barnett et al. 2012). Tagging studies along their coastal habitats have revealed seasonal, sex specific, and long distance (~ 1000 km) movement patterns within their oceanic regions (Ebert 1996, Barnett et al. 2011, Barnett et al. 2012, Stehfest et al. 2014, Stehfest et al. 2015). However, no evidence of transoceanic movement and population connectivity has been reported. Population genetics studies on the species have been limited to the Californian coast, suggesting limited gene flow between two coastal bay populations over a distance >1000 km, between Willapa Bay (Washington) and San Francisco Bay (California) based on nuclear microsatellite data (Larson et al. 2015). To date the global genetics status of the broadnose sevengill shark has not been investigated.

In this study, we use DNA sequence data from the mitochondrial control region (mtCR) and the nuclear ITS2 locus to explore patterns of population genetic population structure in the sevengill shark sampled across three oceanic regions, Eastern Pacific Ocean (EPO), South Atlantic Ocean (SAO) and Oceania. We also use time calibrated phylogenetic reconstructions to investigate the phylogeographic history of the species, and gain insights into historical factors that have shaped contemporary patterns of genetic diversity. Finally, considering the numerous historical synonyms for this species across its geographic range, we discuss our findings in the context of species taxonomy. Findings from this study are expected to enhance our understanding of the population structure of *N. cepedianus* globally and assist with the development of management strategies for this ecologically important but under studied apex-predator.

2.3 Methods

2.3.1 **Tissue collection**

A total of 249 samples were obtained from six countries in three oceanic basins; Eastern Pacific Ocean (EPO), (United States, n = 33 and Peru, n = 22); South Atlantic Ocean (SAO), Argentina, n = 47 and South Africa, n = 42; and Oceania, Australia, n = 65, and New Zealand, n = 40) (*Fig. 2.1*). Tissue samples were obtained as fin clips or muscle punches and preserved in 95% ethanol.

2.3.2 **DNA extraction and sequencing**

Genomic DNA was isolated from tissue samples using the QIAGEN DNeasy Tissue kit (QIAGEN, Inc., Valencia, CA). The entire mtCR plus some flanking DNA was amplified using primers CRF6 (C. Bruels, unpublished, 5' AAGCGTCGACCTTGTAAGTC 3') and DasR2 (V. Richards, unpublished, 5' GCTGAAACTTGCATGTGTAA 3') for all samples. The ITS2 was amplified using previously published primers (Shivji et al. 2002) for a subset of the samples (United States: n = 8, Peru: n = 9, Argentina: n = 10, South Africa: n = 10, Australia: n = 15, and New Zealand: n = 3) as a preliminary analysis. PCR reactions were performed following the protocol outlined in Clarke et al. 2015. In each set of PCR amplifications, a negative control with no genomic DNA was included to check for contamination.

Amplified products were purified using the QIAquick PCR Purification Kit (QIAGEN, Inc.) prior to direct cycle sequencing with BigDye 3.1 Terminator chemistry (Applied Biosystems, Inc., Foster City, CA) on both strands using amplification primers. Sequencing reactions were purified using Dyex 2.0 Spin Kit (QIAGEN, Inc.) and sequenced on an AB3130 genetic analyzer (Applied Biosystems, Inc.). Sequences were aligned with GENEIOUS version 4.04 (Drummond et al. 2008) and alignments were checked and finalized by eye. ITS2 sequences were aligned manually using BioEdit.

2.3.3 **Population genetic analyses**

The number of mtCR haplotypes was identified using ARLEQUIN 3.5 (Excoffier & Lischer 2010) and DnaSP v5 (Labrado & Rozas 2009). To visualize the relationships, clustering, and diversity among haplotypes, a median-joining (MJ) network of haplotypes was constructed (Bandelt et al. 1999) using the program PopART (http://popart.otago.ac.nz). A statistical parsimony network analysis was conducted using TCS version 1.21:3 software (Clement et al. 2000) using mtDNA sequences for all samples. This program joins haplotypes into a network after calculating the 95% probability of a parsimonious connection between haplotypes.

Diversity indexes such as Haplotypic (h) and nucleotide (π) diversity, as well as the number of polymorphic sites (S) were calculated using ARLEQUIN 3.5 and DnaSP v5. The program jModelTest (Darriba et al. 2012) was used to determine the best fit model of sequence evolution and the nearest available model in ARLEQUIN 3.5 was used. An analysis of molecular variance (AMOVA) under the Tamura-Nei (TN) nucleotide evolution model (Tamura & Nei 1993), a commonly used model in assessing population structure in sharks, was performed to assess genetic population structure in ARLEQUIN 3.5. The TN model accounts for the differences in substitution rates between nucleotides, unequal nucleotide frequency and assumes an equal substitution rate among sites. Pairwise estimates of Φ_{ST} and conventional F_{ST} were obtained using ARLEQUIN 3.5 and ran with 1000 simulations and significance level set at $P< 0.05$. The number and location of genetically distinct populations was inferred from the patterns of pairwise genetic differentiation and evolutionary relationships. The neutrality tests Tajima's D (Tajima 1989) and Fu's FS (Fu 1997) were used to detect population expansion and were calculated using MEGA6 (Tamura et al. 2013) and ARLEQUIN 3.5. Both Fu's FS and Tajima's D were calculated as a deviation from neutrality possibly attributed to selection and/or population size changes, with significance level tested at $P< 0.05$ for 1000 permutations. Negative (significant) Fu's FS and Tajima's D values can be interpreted as a signal of purifying selection or demographic expansion. To determine the genetic differences between regions a mismatch distribution analysis was conducted using ARLEQUIN 3.5 and DnaSP v5. To

determine the phylogenetic relationships among haplotypes a maximum likelihood phylogenetic tree was constructed based on the Tamura-Nei evolutionary model, with 1000 bootstrap repeats, using the program MEGA6.

2.3.4 **Phylogenetic analyses and divergence time estimation**

Phylogenetic reconstructions were performed using Bayesian Inference (BI) methods implemented in BEAST 2.3.0 (Bouckaert et al. 2014). General Time Reversible model (Tavaré 1986) with gamma distribution of rates across sites (GTR + G) was selected as the best fit model of evolution for each of the mtDNA and nuclear genes, based on Akaike Information Criteria (AIC) (Akaike 1981) implemented in JMODELTEST v.0.1.1 (Posada 2008). Operators were auto-optimized, and five independent Markov Chain Monte Carlo (MCMC) runs were performed using a Yule (speciation) tree-prior, each running for 5×10^6 generations, sampling every 10,000 states. Log files were examined with TRACER v.1.5 (Drummond & Rambaut 2007) to ensure that runs were sampling from the same posterior distribution, to determine appropriate burn-in, and to ensure that effective sample sizes (ESSs) of parameters of interest were greater than 1,000. Tree files of independent runs were then combined with LOGCOMBINER v.2.1.3 (Drummond et al. 2012), discarding the first 20% of trees as burn-in. The maximum clade credibility (MCC) tree was recovered from a sample of 10,000 posterior trees, and branch support was annotated, using TREEANNOTATOR v.2.1.3 (Drummond et al. 2012). All analyses started with a random starting tree and seed with no root specified. Sequence data from Trisetacus species was used to estimate the root of the mitochondrial gene tree.

To test the timing of diversification between sevengill shark mitochondrial lineages, the gene tree was time calibrated with divergence times of nodes being inferred from 95% highest posterior density (HPD) intervals. The time dimension of the analyses was calibrated by fixing the mean substitution rate to 1.2% per million years (clock rate 0.012), calculated as the mean rate per lineage based on previous estimates for MtCR from a variety of fish and shark species

(Martin et al. 1992, Donaldson & Wilson 1999, Duncan et al. 2006). Substitution rates were set in BEAUti 1.7.3 (Drummond et al. 2012), and TRACER was then used to obtain parameter estimates for time to the most recent common ancestor (tMRCAs) for nodes within the gene tree.

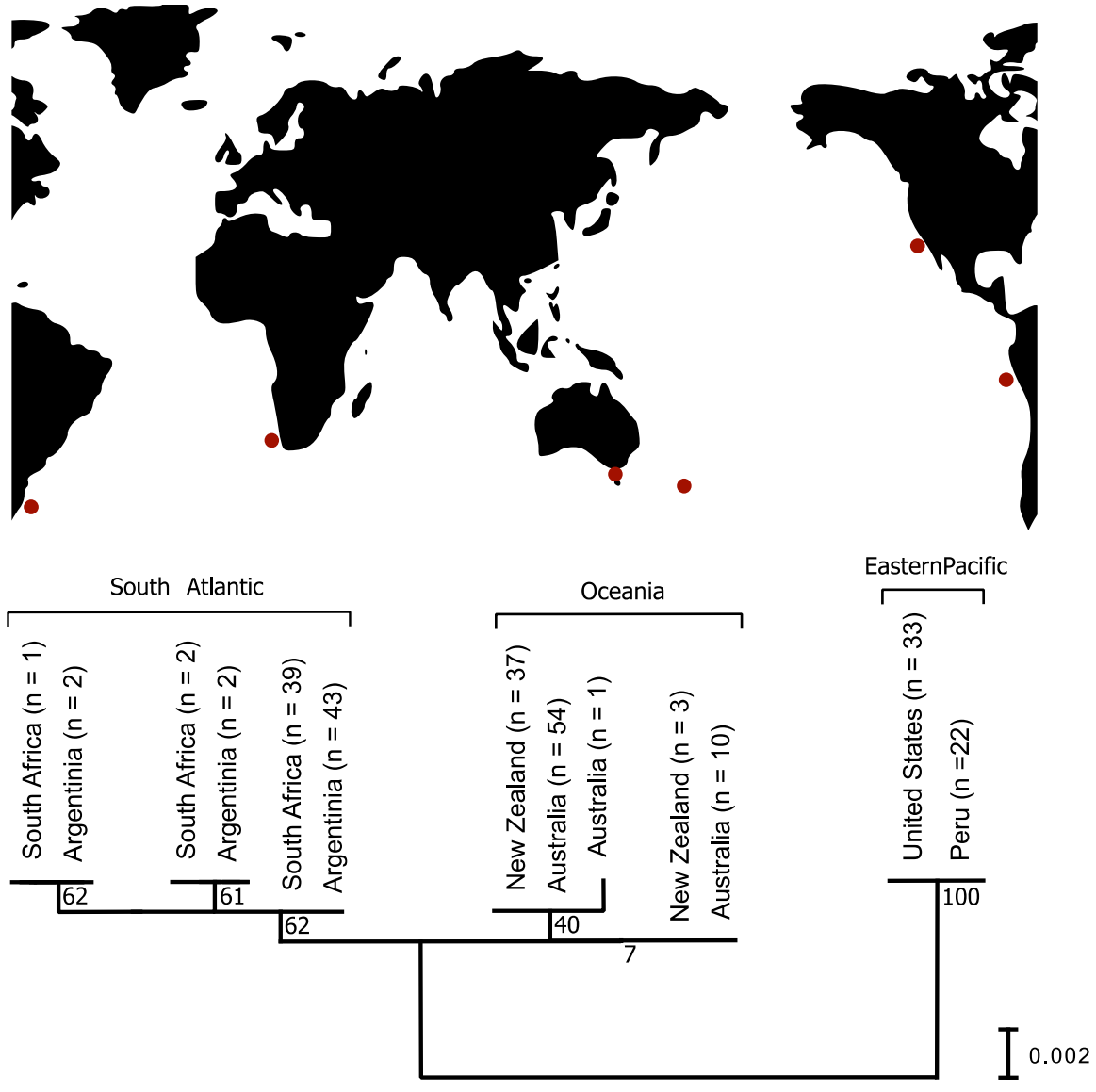

Figure 2.1 – Map showing collection locations for broadnose sevengill shark (*N. cepedianus*). Collection locations include South Africa, Argentina, New Zealand, Australia, United States and Peru. Unrooted maximum likelihood

phylogenetic tree, using Tamura-Nei model at 1000 bootstrap replications. Numbers represent bootstrap values. Tree created in MEGA6

2.4 Results

2.4.1 Genetic diversity and population structure

In this study 812 bp of the mtCR were sequenced in a total of 249 *N. cepedianus* from nine collection locations (grouped into six locations), in three oceanic regions: the EPO (United States (California) and Peru), SAO (Argentina and South Africa), and Oceania (Australia (Tasmania, South Australia, Victoria) and New Zealand (northern and southern New Zealand)) (*Fig. 2.1*). There were a total of seven mitochondrial haplotypes (referred to as Hap_1 – 7, *Appendix 7.3*) defined by 15 polymorphic sites (*Table 2.1* & *Table 2.2*). The overall haplotype diversity (h) was 0.709 and nucleotide diversity (π) was 0.00591 (*Table 2.1*). The topology of the median joining network (*Fig. 2.2*) consisted of two highly divergent lineages. In the Eastern Pacific, individuals sampled from the United States and Peru shared a single haplotype (Hap_1) that was separated from all the other haplotypes by 11 mutation steps. Although the remaining haplotypes (Hap_2 – 7) segregated geographically between the South Atlantic Ocean (Argentina and South Africa) and Oceania (Australia and New Zealand), these two regions were only separated by a single mutational step. The statistical parsimony network analysis indicated similar stratifications of regions, with a decisive separation between the Eastern Pacific and other regions (*Fig. 2.3*). The haplotype frequency for each group is shown in *Table 2.2*. Similarly, a statistical phylogenetic reconstruction using the Maximum likelihood phylogenetic tree revealed two clades, separating the Eastern Pacific from the South Atlantic and Oceania lineages (*Fig. 2.1*).

Results from AMOVA showed strong and significant genetic differentiation between the oceanic regions (global F_{ST} =0.9789, $P < 0.0001$), with pairwise ΦST indicating high levels of significant differentiation between all oceanic regions (Eastern Pacific, South Atlantic Ocean,

Oceania) ($P < 0.0001$). In contrast AMOVA indicated no significant differences among collection locations within regions ($P = 0.479 \pm 0.01$).

Demographic summary statistics (*Table 2.1*), such as the Fu's FS, were negative and not significant for the oceanic regions, however, values for the Eastern Pacific region could not be calculated as a result of no differentiation present within the region. Tajima's D values were also negative and non-significant, $P< 0.001$, at 1000 bootstraps. The mismatch distribution graph was stochastic and multimodal, the Sum of Squared Deviation (SSD) and Harpending's Raggedness Index (HRI) p-values were non-significant from that expected under population expansion for South Atlantic, p(Sim >= Obs) = 0.395 and 0.647 respectively, and Oceania, p(Sim >= Obs) = 0.348 and 0.630 respectively, $P < 0.05$. The difference between $\Theta 0$ and $\Theta 1$ was small for all regions, a difference of 0.185 and 0.326 for South Atlantic and Oceania respectively. The τ-values were 3 for both the South Atlantic and Oceania, which may be an indication of a stationary population.

In contrast, the ITS2 data was highly conserved. The only difference was a variation in the number of repeats present in a dinucleotide repeat motif (bp 276 to 297 of the 776 bp alignment). All individuals from the Californian region had 6-8 repeats, while 10 repeats were present in the Peruvian sequence and the Atlantic/Oceania region. As a consequence of this lack of locus specific variation, the ITS2 data was not used for population genetic or phylogenetic analyses.

2.4 Results

Table 2.1 – Population genetics statistics for regions and collection locations. Number of samples (N), haplotype number (H), number of polymorphic sites (S), haplotype diversity (h), nucleotide diversity (π), Harpending's raggedness Index, SSD and test of neutrality (Tajima's D & Fu's Fs) for the broadnose sevengill shark (*N. cepedianus*) mitochondrial DNA control region.

		Genetic diversity indicies					Neutrality Tests		Mismatch Analysis	
Region	Collection location	*N*	*H*	*S*	*h*	π	Tajima's *D*	Fu's *FS*	Harpending's Raggedness Index	SSD
Eastern Pacific Ocean (EPO)	United States	33	1	0	0	0	0	0	0	0
	Peru	22	1	0						
	Pooled EPO	***55***								
South Atlantic Ocean (SAO)	Argentina	47	2, 3, 4	2						
	South Africa	42	2, 3, 4	2	0.1496 ± 0.05	0.000189 ± 0.000296	-0.96493	-1.6086	0.51591	0.00057
	Pooled SAO	***89***								
Oceania	Australia	65	5, 6, 7	2	0.2357 ± 0.05	0.000295 ± 0.00038	-0.57551	-0.7271	0.33526	0.00427

	New Zealand	40	5, 6	1						
	Pooled Oceania	*105*								
Total samples		**249**	**1-7**	**15**	**0.709 ± 0.012**	**0.00591 ± 0.00038**	**2.35208**	**10.145**	-	-

2.4 Results

Table 2.2 – Frequency of haplotype in the collection locations, haplotype name (Hap_1 – 7), number of samples (N), for the broadnose sevengill shark (*N. cepedianus*) mitochondrial DNA control region.

Haplotype *(N)*	Collection location					
	United States (33)	*Peru (22)*	*Argentina (47)*	*South Africa (42)*	*Australia (65)*	*New Zealand (40)*
Hap_1 *(55)*	1	1	-	-	-	-
Hap_2 *(82)*	-	-	0.915	0.929	-	-
Hap_3 *(3)*	-	-	0.0426	0.0238	-	-
Hap_4 *(4)*	-	-	0.0426	0.0476	-	-
Hap_5 *(91)*	-	-	-	-	0.831	0.925
Hap_6 *(13)*	-	-	-	-	0.154	0.075
Hap_7 *(1)*	-	-	-	-	0.0154	-

Table 2.3 – Conventional Pairwise F_{ST} values (below diagonal) and p-values (above diagonal, + represents statistical significance with p-value < 0.05) for broadnose sevengill shark (*N. cepedianus*) across three regions. Significant results denoted in Bold.

	Eastern Pacific	South Atlantic	Oceania
Eastern Pacific	0	+	+
South Atlantic	**0.90885**	0	+
Oceania	**0.85028**	**0.80442**	0

Table 2.4 – Pairwise Φ_{ST} values (below diagonal) and p-values (above diagonal, + represents statistical significance with p-value < 0.05) for broadnose sevengill shark (*N. cepedianus*) across three regions. Significant results denoted in Bold.

	Eastern Pacific	South Atlantic	Oceania
Eastern Pacific	0	+	+
South Atlantic	**0.99228**	0	+
Oceania	**0.98698**	**0.8989**	0

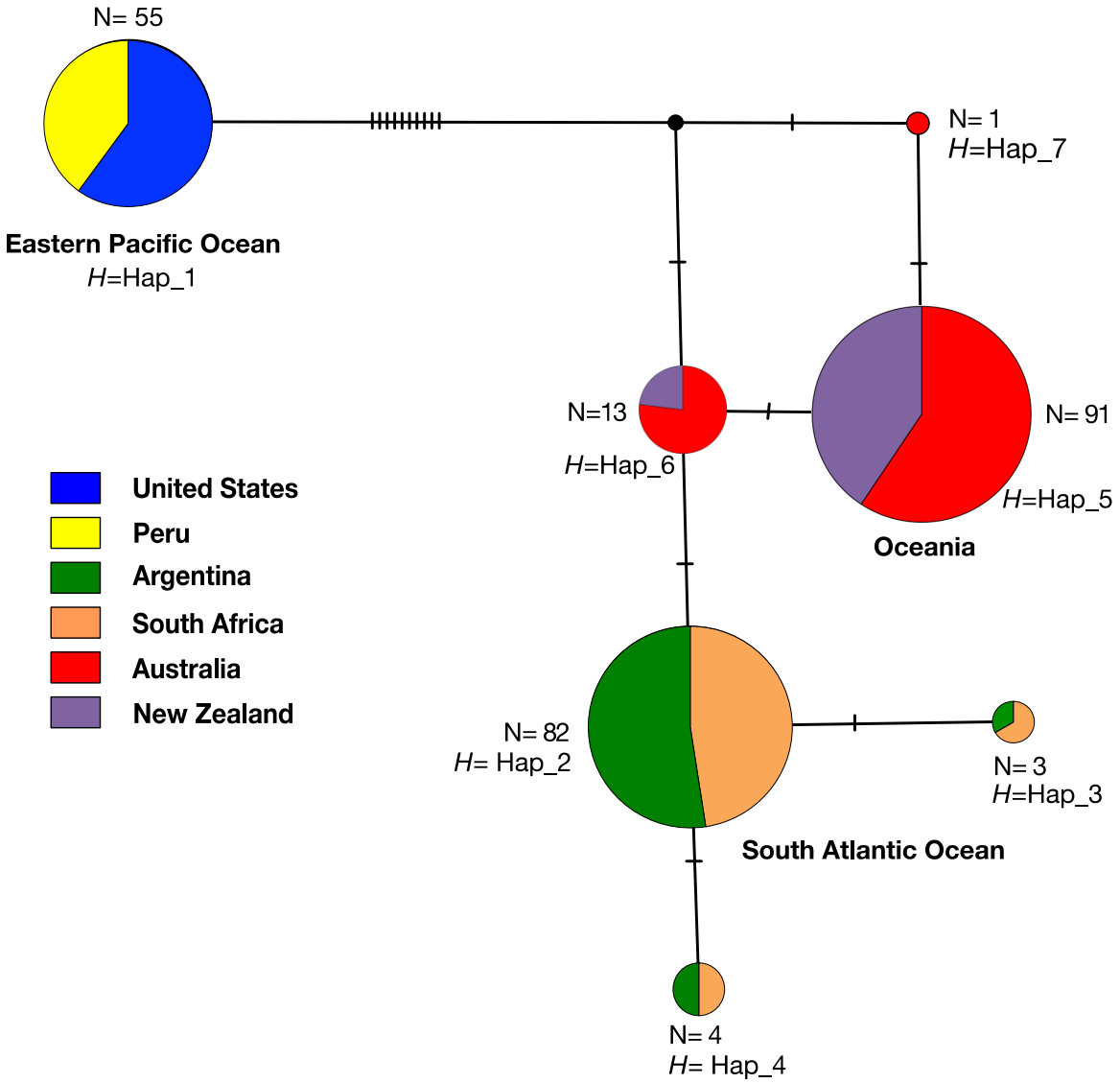

Figure 2.2 – Median-joining network of mtCR haplotypes for *Notorynchus cepedianus*. Circles represent individual haplotypes with circle size proportional to haplotype frequency, connection lines indicate one base pair difference and breaks indicate inferred un-sampled haplotypes. Collection locations are as follows: United States (Blue), Peru (Yellow), Argentina (Green), South Africa (Orange), Australia (Red), and New Zealand South (Purple). Numbers of samples per circle size (N), haplotype designation (*H*)

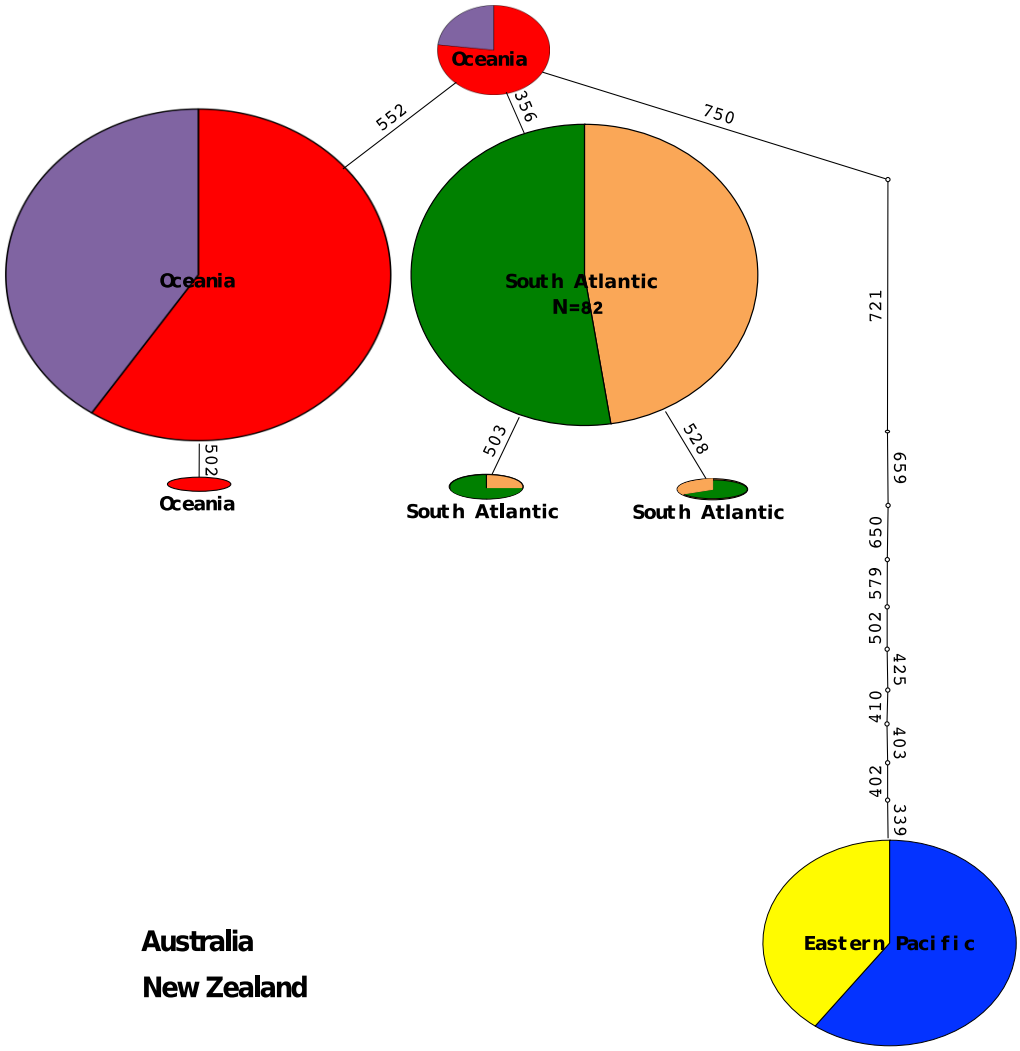

Figure 2.3 – Broadnose sevengill shark 95% parsimony network. Circles, ovals and square represent haplotypes of the respective regions. Size of the circles and ovals correspond to haplotype frequency and nodes indicate inferred un-sampled haplotypes. The number of samples is represented by "N".

2.4.2 **Phylogenetics and divergence**

Bayesian Inference phylogenetic reconstruction indicated a pattern of paraphyly and strong support for three distinct monophyletic clades representing the Pacific, Atlantic, and Oceania regions (Posterior Probability (PP) > 0.9). A fourth moderately supported (PP = 0.8) clade consisting of haplotypes from the Oceania region was also revealed, indicating potential paraphyly of the Oceania population. A sister relationship between the Atlantic and Oceania clades gained strong statistical support (PP = 1.0), while the relationships among the three clades were not fully resolved. A basal position of the Pacific Ocean clade was also highly supported (PP = 1.0). Time calibrated branch divergences indicate that all four clades diverged from a common ancestor approximately 0.55 Mya (95% HPDs = 0.34 – 0.72), with the Atlantic and Oceania clades sharing a more recent common ancestor approximately 0.28 Mya (95% HPDs = 0.20 – 0.37) before the present day. The timing of these events coincides with the mid to late Pleistocene.

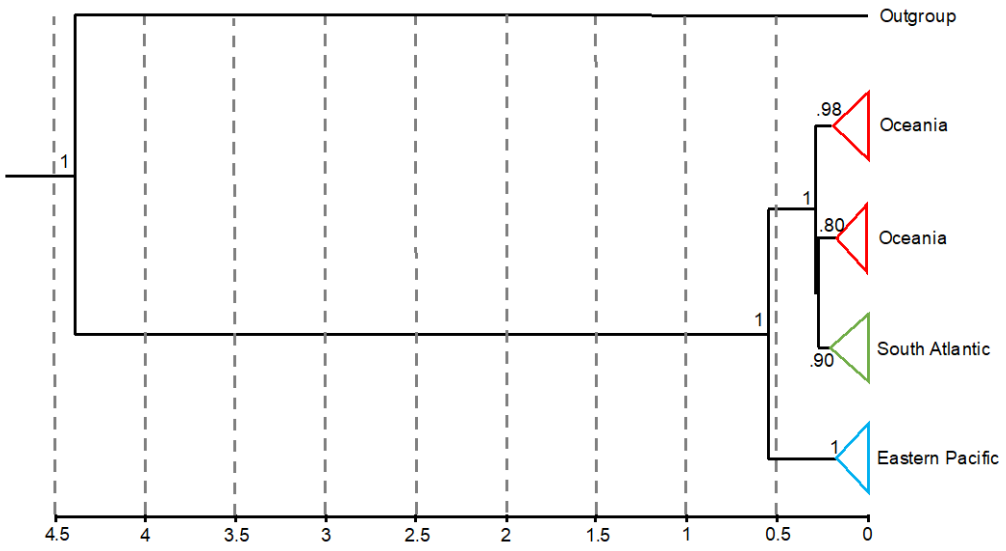

Figure 2.4 – Time calibrated Bayesian Inference phylogenetic reconstruction of relationships among broadnose sevengill shark mitochondrial control region haplotypes. Nodal support values provided represent Bayesian posterior probabilities (>0.8), and estimated tMRCAs with 95% highest posterior density intervals (illustrated by

purple bars at branch nodes) are provided, with the scale provided in millions of years. The "Outgroup" consists of the genus *Hexanchus* (sister taxa).

2.5 Discussion

2.5.1 **Genetic structure**

Significant biogeographic structuring of *N. cepedianus* was observed among the three oceanic regions, EPO, SAO and Oceania. While connectivity was observed within regions, a clear pattern of transoceanic isolation was evident, with statistically significant differentiation among all regions, and the strongest differentiation being between the EPO and the other two regions. Haplotype networks and Bayesian Inference phylogenetic reconstructions further reiterated the strong subdivision between oceanic regions, demonstrating a lack of shared haplotypes between regions, strong statistical support for the monophyly of regional phylogenetic clades, and a basal and most divergent positioning of the EPO. Combined, these results indicate significant genetic structuring and a lack of historical and contemporary gene flow among oceanic regions. Similarly, significant genetic differentiation has been described for a variety of shark and ray species between the Atlantic and Indo-Pacific oceanic basins (Naylor et al. 2012). Geographic barriers such as the Indo-Pacific Barrier (IPB) in addition to soft barriers such as upwelling, currents, large distances, and geological processes may have played a role in restricting gene flow between these oceanic regions and driving the observed patterns of genetic differentiation.

Mismatch distribution analysis Harpending (1994), showed a ragged and erratic distribution (multimodal distribution), which is indicative of a population at demographic equilibrium over time. Harpending's index and SSD values (*Table 2.1*) also suggest a stable global population for the broadnose sevengill shark. Neutrality tests (Tajima's *D* and Fu's *F*) are indicative of purifying selection or possible population expansion. However, considering the mismatch distribution results, the neutrality results are most likely symptomatic of negative selection

leading to a stabilization selection in the population, which can lead to a decrease in genetic diversity. Negative selection or purifying selection is the selective removal of deleterious alleles within a population and results in the loss of variations that may arise (stabilising selection). Populations with low genetic diversity are more susceptible to processes such as background selection, which is the inadvertent elimination of non-deleterious alleles in close proximity to deleterious alleles, indicative of strong negative selection.

N. cepedianus showed low haplotype structure but levels of genetic diversity ($H = 7$, $h = 0.709$, $\pi = 0.00591$) that are similar to other coastally associated shark species, e.g. *Carcharhinus limbatus:* $H = 37$, $h = 0.843$, $\pi = 0.00413$ (Keeney & Heist 2006); *Carcharhinus leucas:* $H = 14$, $h = 0.76$, $\pi = 0.0028$ (Karl et al. 2011); genus *Negaprion:* $H = 11$, $h = 0.78$, $\pi = 0.00585$ (Schultz et al. 2008). This was unanticipated considering it is generally expected that older taxonomic groups, such as the broadnose sevengill shark (Tanaka et al. 2013), should have higher genetic diversity than their younger counterparts as older groups have had more time to accumulate genetic variation. Low genetic diversity is often a result of reductions in population size, leading to a loss of diversity through genetic drift and bottleneck processes. *N. cepedianus* were targeted across their geographical range, including California (San Francisco Bay), South Africa, Namibia, Argentina, Peru and Australia, mainly from the 1930s – 1980s (Ebert 2001, Cedrola et al. 2009, Barnett et al. 2012, Larson et al. 2015, De Wysiecki et al. 2018). In South Africa, exploitation levels were considered to be low, on average less than 10t dressed weight reported per year (da Silva et al. 2015). In Peru *N. cepedianus* remain part of the elasmobranch fishery, while in Australia they are a bycatch of the southern finfish and shark fishery, as well as in recreational fishing (Barnett et al. 2012). However this species is considered to be of low economic value and is no longer actively targeted across most of its distribution. Studies have however shown this species to be sensitive to fishing pressure, as a result of their life-history traits, where targeted. (Smith et al. 1999, De Wysiecki et al. 2018). Considering the varying levels of regional exploitation recorded for this species, it is unclear as to the extent of the impact fishing has had on this species populations structure. Thus, further study is required to ascertain the exploitation rates of this species at regional levels to accurately determine population effects. A possible explanation for this species low genetic diversity, particularly in

the Pacific populations, which was only represented by one CR haplotype, may be the low evolutionary rates of mitochondrial DNA (mtDNA) for sharks, at least six to eight times slower than in mammals (Martin et al. 1992).

In North America, *N. cepedianus* are distributed from British Columbia (Canada) to the tip and bay of Baja California (Mexico), distribution reestablishes in South America from Columbia, along the Pacific coast, up to southern Brazil in the South Atlantic. The strong genetic similarities between populations within regions, particularly the lack of genetic differentiation between California and Peru, is puzzling considering the large expanses of water (>2,000 km) separating the various populations. Although no evidence of transoceanic movements has been documented, this species moves between coastal bays (males up to ~1000 km, in Australia) (Barnett et al. 2012, Stehfest et al. 2014), and a female travelled >1800 km from Willapa Bay, Washington area to Mission Beach, California (Williams et al. 2012). Adult movement across the equator would be unexpected considering the thermal preferences of this species, thus it is unclear why there is such a high level of genetic similarity between these two Eastern Pacific regions. Plausible explanations could include, some remnant historical evolutionary connection in combination with isolation effects and slow mutation rates. *N. cepedianus* have been recorded to depths of 550m (Anderson et al. 1998). This may enable them to use of deep cold-water currents to bridge the equatorial barrier and allow for physical mixing between the two Eastern Pacific locations. Considering the distance issue, it is unclear whether any physical mixing between populations from California and Peru, even given the possible stepping stone populations in Baja California and Columbia, is likely. Similarly considering the barriers, such as large expanse of water separating the eastern and western Atlantic populations (Argentina and South Africa) and the populations in Oceania (Australia and New Zealand), currents, species movement capabilities and behavior (site fidelity), it is most likely that evolutionary and possible anthropogenic processes are responsible for the low genetic diversity and strong connectivity within these regions. Islands such as Tristan de Cunha, Inaccessible, Nightingale and Gough in the south Atlantic could act as a bridge between the eastern and western Atlantic population. More intensive sampling of these areas between main regions, or inclusion of samples from the Tristan de Cunha, Inaccessible, Nightingale and Gough in the south Atlantic

will facilitate a better understanding of stepping stone populations that may allow for sufficient gene flow to prevent differentiation between these regions. Additionally, further long distance spatial movement studies are required to delineate the boundaries of this species dispersal capacity.

Site fidelity behavior has been observed for *N. cepedianus*, with seasonal movement into and out of coastal bays during the spring-autumn and winter months respectively (Ebert 1996, Barnett et al. 2010c, Williams et al. 2011, Barnett et al. 2012, Williams et al. 2012). Sexual segregation and movement has also been observed for this species with some females exhibiting site fidelity to particular coastal areas and males traveling longer distances between coastal bays (Lucifora et al. 2005, Barnett et al. 2011, Stehfest et al. 2014, Stehfest et al. 2015). Biological barriers such as migratory, philopatric and site-fidelity behavior have a strong influence on movement. Migratory behavior, whether reproductively or resource driven, can result in genetic connectivity across large distances in highly vagile species such as the great white shark (Andreotti et al. 2016) and whale sharks (Schmidt et al. 2009). In contrast, philopatric behavior can confine movement to specific localities and restrict genetic mixing. This pattern has been observed in coastally associated sharks species and has a strong influence on genetic structure within regions (Keeney & Heist 2006, DiBattista et al. 2008, Schultz et al. 2008, Chin et al. 2016). In lemon sharks (*Negaprion brevirostris*), across their western Atlantic distribution, female philopatric behaviour is responsible for restricted gene flow within the northern hemisphere (fine scale), while distance and historical processes influenced population differentiation between the northern and southern hemispheres (large scale) (Ashe et al. 2015). Bull sharks (*Carcharhinus leucas*) exhibit similar genetic and phylogeographic patterns through restricted maternal gene flow as a result of natal philopatry (Karl et al. 2011). Our study indicates that inter-populations (across regions) are strongly affected by historical events and biogeographical barriers (IPB, equator, distance, currents and temperature) while intra-populations studies (within regions) (Larson et al. 2015) suggest that genetic diversity is shaped by biological barriers such as philopatry and site-fidelity.

The type specimen of this species was sampled in Tasmania, Australia, originally described as

Squalus cepedianus, later renamed *Notorynchus cepedianus* (Péron 1807). Though numerous designations for this species were created across its geographic range, over subsequent years, a synopsis of all names was established using comparative morphological examinations of specimens (Compagno 1984). It is widely known that there are inherent limitations to morphology-based identification as it can overlook such occurrences as phenotypic plasticity and cryptic speciation, which is common in many species (Hebert et al. 2003, Bickford et al. 2007). Additional morphological keys are often only valid for certain life stages and require a certain level of expertise, which also leads to frequent misidentification. The increased utilisation of, and advancements in, genetic techniques have further reiterated the need for multifaceted approaches to species classification. As a result species classification has become a fluid field with adjacent concepts such as the Genetic Species Concept (species are genetically isolated, but not reproductively isolated, GSC) (Baker & Bradley 2006) and the Phylogenetic Species Concept (irreducible group with members derived from descendants of a common ancestor and possess a combinations of apomorphy, PSC) (Wheeler 1999), challenging traditional understandings. There are several examples of where traditional species concepts, such as Morphological Species Concept (MSC), have failed to reveal hidden diversity within marine species, e.g. marine mammals (Baker & Bradley 2006), corals (Schmidt-Roach et al. 2014) and fish (Martinez-Takeshita et al. 2015). Parsimony network analysis has been used as a phylogenetic tool for detecting cryptic and undescribed species (Hart & Sunday 2007, Chen et al. 2010). This method allows for the differences between species to be determined by the length of the branches, with long branches indicating differences between taxa and short branches, within taxa (Pons et al. 2006, Hart & Sunday 2007). Our parsimony network showed a clear delineation of the Eastern Pacific from the other regions. A study by Naylor et al. (2012), using the NADH dehydrogenase subunit 2 (NADH2) region, showed that even within the sister genera species (*Hexanchus nakamurai*), the genetic variation between specimens from the Indian and Pacific Ocean is similar to that observed between the Eastern Pacific and Oceania samples for *N. cepedianus*. Thus, considering the complexity associated with classification a broader outlook may be needed to provide more definitive answers.

Bearing in mind that the traditional MSC originally used to define this species, may have overlooked clandestine diversity within this group, a more modern concept, such as the aforementioned species concepts may be more suitable and informative. In particular the PSC, given its indistinguishable morphological characteristics, and the GSC, given the strong genetic distinction observed between regions in this study. As the oldest Eastern Pacific description, to our knowledge, was made in California for *Notorynchus maculatus* Ayres 1855, we would suggest this as a possible valid representation of this taxon. However, further genetic research, particularly within regions, is required to unravel the full depth of diversity within this species, provide additional support to this proposal, and possibly identify further speciation within this genus.

2.5.2 **Phylogeography**

Understanding phylogeography patterns can provide information on the historical processes that shape a species' contemporary geographical distributions. This study revealed four distinct clades (Eastern Pacific, South Atlantic and two clades within Oceania) divergent from a common ancestor approximately 0.55 Mya. The Atlantic and Oceania clades share a more recent common ancestor approximately 0.28 Mya. Within Oceania there is a sub-division, possibly representing Australian and New Zealand populations. These divergences occurred within the mid-Pleistocene epoch approximately 781,000 to 126,000 years ago, representing the most recent ice age. During this time there were cycles of glacial (cold) and interglacial (warmer) periods which included; Cromerian interglacial (620,000 years ago), Kansan glaciation (450,000 years ago), Hoxnian interglacial (380,000 years ago) and the current interglacial period since approximately 11,000 years ago (Avise 2000). Glacial cycles affected temperate zones primarily through coastal habitat disruption (Bowen et al. 2016). Near shore ecosystems were lost when glaciers expanded onto the continental shelves, while during warmer period coastal ecosystems were restored/increased, encouraging colonisation and population expansion. The phylogeographic partitioning and low global genetic diversity exhibited by *N. cepedianus* could be a result of these glacial cycles, which may have led to

isolating effects (biogeographic barriers) and population fluctuations (decline and expansion). *N. cepedianus* show dependence on coastal habitats through key feeding and possible breeding areas (nursery/pupping) (Barnett et al. 2012), thus the loss or restoration of these areas may have resulted in fluctuating population decline and expansion. Low global genetic diversity may be as a result of founder effects, if the ancestral colonizing population to the different oceanic zones was small. Similarly, silky sharks were shown to have globally divergent lineages, between the Atlantic and Indo-Pacific originating during the Pleistocene epoch (Domingues et al. 2017).

One of the most prominent marine biogeographic barriers is the large expanse of water across the Pacific and Atlantic oceans (Lessios et al. 1998). A species ability to overcome this barrier in temperate zones is dependent on dispersal capability, temperature tolerance and climate history (Bowen et al. 2016). Many marine species are incapable of crossing large expanses of water, and as a result, clear biogeographic patterns have emerged delineating spatial boundaries between the east/west and north/south regions of the Pacific and Atlantic oceans. Temperate regions tend to have fewer "stepping stone areas" compared to tropical regions, which can restrict an organism's ability to cross large expanses of water. For example, many islands, including the Hawaiian archipelago, act as a bridge between the east and west tropical Pacific, allowing some species to maintain their population connectivity. Many marine taxa such as fish, cetaceans and marine turtles display these phylogeographic patterns (Avise et al. 2016).

2.6 Conclusion

This is the first study to investigate the genetic diversity and phylogeographic patterns of the broadnose sevengill shark *Notorynchus cepedianus* across its global distribution. Overall our results showed three distinct genetic clades among oceanic regions, EPO, SAO and Oceania, with the Eastern Pacific population being particularly distinct from the other oceanic regions.

Although differentiation between the regions was high, our data suggest that the divergence may not be sufficient enough to warrant reclassification as separate species. However, considering the changing nature of the taxonomic and genetic fields further evaluations are recommended to determine the global categorisation of *N. cepedianus* in the future.

Within oceanic regions genetic diversity was low, indicative of high genetic connectivity within regions. Additionally, *N. cepedianus* exhibit phylogeographic patterns similar to other large coastal sharks, with large-scale genetic patterns predominantly influenced by historical and geographic barriers, whereas finer-scale patterns within regions were likely affected by biological barriers, such as site-fidelity and philopatry. This study shows that *N. cepedianus* is not a panmictic species and that populations are divided on an oceanic scale due to behaviour (movement ability) and physical barriers (sea surface temperature, distance), similar to other coastal associated shark species. Movement studies for this species also indicated that individuals have not been shown to travel between ocean basins, reiterating a lack of connectivity between the oceanic regions. Thus, our findings suggest that management and conservation plans for this species needs to be focused within oceanic regions. As no studies on this species has shown movement between countries within regions, i.e. between USA and Peru, Argentina and South Africa and Australia and New Zealand, at this time intra-regional collaborations may not be required and management can be concentrated within countries. This study reiterates the need for further information on fisheries mortality rates, intra-regional genetic structure and movement for each population (within countries), to fully understand the stock structure and identify high-risk populations of *N. cepedianus* across their geographical distribution. Only with this information can the conservation status of *N. cepedianus* be determined and effective management strategies developed and implemented.

3 Genetic connectivity of the broadnose sevengill shark, *Notorynchus cepedianus*, across its range in Australia

3.1 Abstract

Sharks are among the most threatened species on the planet, with one in four species listed as threatened (Musick 2005, Musick & Bonfil 2005, Dulvy et al. 2014). A lack of basic information on the patterns of gene flow and connectivity, both at a global and regional scale, has hindered the effectiveness of management and conservation strategies for many species. The broadnose sevengill shark, *Notorynchus cepedianus,* is an important predator and a common coastal associated species in temperate regions across the globe. Little is known about this species' genetic structure, particularly in the Australian region. Here, we investigated the population genetic structure of *N. cepedianus* in Australia using a Genotyping by Sequencing (GBS) approach. We identified 2544 single nucleotide polymorphisms (SNPs) for 188 individuals, collected from five areas in south-eastern Australia, and included one out-group from South Africa. Genetic diversity was moderated with an overall observed and expected heterozygosity of 0.51 and 0.32 respectively. Analyses of the genetic structure for this species around Australia indicate high levels of connectivity resulting in low levels of genetic differentiation within its range in Australia (F_{ST} = 0.006). The only locations that were significantly differentiated from each other in Australia were samples collected from the southern Tasmanian locations, which were significantly differentiated to Victorian locations. Not surprisingly, the samples from South Africa showed the greatest levels of genetic differentiation from Australian samples (F_{ST} ranged from 0.047 to 0.067). This study provides crucial information on the genetic diversity and patterns of connectivity in this species around Australia, which is important for their management and conservation in this region.

3.2 Introduction

Over the last several decades genetic tools have played a pivotal role in wildlife conservation, assisting in the identification of cryptic species (Baker & Bradley 2006, Bickford et al. 2007, Schmidt-Roach et al. 2014, Martinez-Takeshita et al. 2015), patterns of population structure (Bernard et al. 2016, Bester-van der Merwe et al. 2017), and factors influencing population fitness and environmental resilience (Hoelzel et al. 2006, Schmidt et al. 2009). Modern genomic techniques and technologies have provided unprecedented power for detecting fine scale patterns of genetic structure and improving our understanding of both contemporary and historical patterns of gene flow and demographic histories (Naylor et al. 2012, Narum et al. 2013, Larson et al. 2017). Consequently, the use of genomic data has become a powerful tool for identifying and guiding the management and conservation of discrete populations, which is often necessary for preserving species and maximising their evolutionary potential (Morin et al. 2009, Schindler et al. 2010, Dudgeon et al. 2012, Larson et al. 2014). In the absence of such information, ineffective management of discrete populations can lead to population decline and localised extinctions (Pinsky & Palumbi 2014). Many marine species world-wide are under threat as a result of anthropogenic effects including habitat fragmentation and degradation, pollution and over-exploitation (Halpern et al. 2008). Sharks are among the most threatened species in the world, with an estimated one quarter listed as threatened (Dulvy et al. 2014). While several shark stocks are considered depleted, such as; porbeagle shark (*Lamna nasus*), Northwest Atlantic (Campana & Gibson 2008); blue shark (*Prionace glauca*), North Atlantic (Campana et al. 2006, Campana et al. 2009); basking sharks (*Cetorhinus maximus*), North Pacific (McFarlane et al. 2009); and spiny dogfish (*Squalus acanthias*), North Atlantic and Pacific (Wallace et al. 2009), increased research and information on stock structure, biology (reproduction), behavior (phylopatry) and movement has improved management strategies for some sharks species. Preliminary recovery has been shown for some shark stocks, e.g. spinner, blacktip, sandbar and tiger sharks in some regions, through increased ecological knowledge and fisheries management (Peterson et al. 2017, Simpfendorfer & Dulvy 2017). This emphasises the importance of information on stock structure and biology for the development of efficient management and conservation plans.

Traditionally, widely distributed shark species were believed to comprise of large interconnected and genetically diverse populations spanning large geographic regions (Bernard et al. 2016). However, genetic studies in recent years have provided new insights into patterns of population connectivity among shark populations spanning the world's oceans and suggest that patterns of genetic structuring are highly variable. For example, research on basking shark (Hoelzel et al. 2006) and whale sharks (Schmidt et al. 2009, Castro et al. 2007) have revealed low levels of genetic structuring and panmixia on a global scale. In contrast, white sharks (*Carcharodon carcharias*) have been shown to consist of distinct populations according to oceanic regions, with genetically different populations occurring in the northwest Atlantic, southern Africa, Mediterranean and Pacific (O'Leary et al. 2015). Similarly, genetic structuring among oceanic regions has been shown to occur in tiger shark (*Galeocerdo cuvier*) (Bernard et al. 2016), oceanic whitetip shark (*Carcharhinus longimanus*) (Camargo et al. 2016), silky shark (*Carcharhinus falciformis*) (Clarke et al. 2015) and pelagic thresher sharks (*Alopias pelagicus*) (Cardeñosa et al. 2014). Furthermore, evidence of fine-scale genetic structuring within major oceanic regions has been shown for some sharks in the *Carcharhinus* genus such as the dusky (*Carcharhinus obscurus*), sandbar (*Carcharhinus plumbeus*) (Geraghty et al. 2014), and spinner shark (*Carcharhinus brevipinna*) (Geraghty et al. 2013) along the eastern coast of Australia, with marginal delineations between populations from the northern and eastern coast. Patterns of fine-scale structuring are suspected to be influenced by philopatric behaviour towards particular nursing or pupping areas, ocean depth, currents and temperature, and are thought to be responsible for regional population subdivisions and genetic structuring (Bernard et al. 2016).

The broadnose sevengill shark, *Notorynchus cepedianus,* (hereafter referred to as the "sevengill shark") is a globally distributed, temperate and coastally associated apex-predator. The global conservation status of this species is listed as "Data Deficient" as a result of major gaps in knowledge about stock structure, connectivity and early life-stages, particularly with respect to movement, habitat use and behaviour (Barnett et al. 2012). Similar to other large bodied shark species, their low fecundity makes them particularly vulnerable to anthropogenic factors such

as over-exploitation and habitat degradation. Regional studies have focused on movement, feeding and reproductive behaviour (Barnett et al. 2012, Williams et al. 2012, Awruch et al. 2014, Stehfest et al. 2014). However, studies on this species' global genetic structure and connectivity are limited. Results of Chapter 2, using traditional genetic markers, suggest strong genetic differentiation between the oceanic regions eastern Pacific, Atlantic and Oceania. A regional study along the eastern coast of the US indicated some genetic differentiation between sevengill shark populations from two coastal bays, with evidence of some mixing (Larson et al. 2015). In Australia, this species can be found in south-eastern region along the coasts of South Australia, Victoria, Tasmania and New South Wales, however, no genetic studies on populations in these regions have been conducted to date.

Currently there are no management plans for *N. cepedianus* in Australia, however they are common by-catch (Zhou et al. 2007, Cedrola et al. 2009, Zhou et al. 2009, De Wysiecki et al. 2018) and recreational fished species. Information on regional population genetic structure can provide information on the stock structure of this species across jurisdictional boundaries for the establishment of management strategies for this shark species. The use of SNPs has been shown to improve the deciphering of fine-scale population structure by revealing previously unexplored genomic regions, providing more accurate estimates of population genetics and elucidating evolutionary patterns compared with more traditional marker systems where differences were undetected (Benestan et al. 2015). Consequently, deciphering population structures at small scales by modern sequencing techniques are highly informative, providing vital guidance to develop effective management strategies. Conservation and management of several marine species has been improved by the information provided by these methods, such as Chinook salmon (*Oncorhynchus tshawytscha*) (Larson et al. 2014), American lobster (*Homarus americanus*) (Benestan et al. 2015), marine mammals (Lah et al. 2016), and the Galapagos shark (*Carcharhinus galapagensis*) (Pazmiño et al. 2017)

This study investigates patterns of gene flow and genetic structure among broadnose sevengill shark populations from south-eastern Australia. Using a panel of genome wide SNP markers derived from a reduced genome representation sequencing method we compare allele

frequencies among sharks sourced from 5 locations across the south-eastern Australian coastline. Our findings will provide fundamental information on the Australian stock structure of *N. cepedianus* and the implications for future management and conservation of this species in Australia is discussed.

3.3 Methods

3.3.1 Sample collection and DNA extraction

A total of 190 fin clip tissues biopsies (approximately 2-3mm) were collected for genetic analysis. South-eastern Australian samples were collected by the Australian Fisheries Management Authority (AFMA) between 2007 and 2015 from four locations, including, Victoria (VIC, n = 9), South Australia (SA, n = 4), northern Tasmania (NTAS, n = 23), and south-eastern Tasmania (Derwent estuary Hobart and Norfolk Bay; STAS, n = 7). Samples from Port Phillip Bay (PPB, n= 118) were collected during acoustic tagging operations between 2014-2015 (see *Chapter 4*). South African samples were collected between 2013 – 2015 from the south-west coast (Atlantic Ocean), and provided by Dr. Alison Kock, Cape Research Centre, South African National Parks. Shark tissue samples were preserved in 70-100% ethanol, DSMO or frozen at -20°C until analysis.

3.3.2 Library preparation and sequencing

Total genomic DNA was extracted from 10mg of muscle tissue using the Qiagen DNeasy 96 Blood and Tissue Kit (Venlo, Limburg, NL), and reduced representation genome libraries were prepared with a modified genotyping by sequencing (GBS) protocol of Elshire et al. (2011). Three hundred nanograms of genomic DNA from each individual was digested in 20 μL reaction containing four units of the restriction enzyme ApeKI for 2 h at 37 °C. Digestion

products were then ligated to modified P1 and P2 adapters with unique barcode combinations to allow for subsequent multiplexing of all individuals. Fifty µL ligations were performed containing the enzyme digested DNA, 1.125 ng of P1 and P2 adapters, 400 units of T4 ligase and T4 buffer (New England Biolabs, Beverly MA, USA). Ligations were incubated at 16 °C for 90 min followed by a 30 min of denaturation at 80 °C. Adapter ligated DNA fragments were purified using a Qiagen MinElute PCR purification kit (Redwood City, CA, USA), eluted in 20 µL of ddH20 and subsequently used for PCR amplification. Fifty µL PCRs were performed using MyTaqTM HS Mix (Bioline, Taunton, MA, USA), and containing 0.2 µL each of Illumina Dual Index Sequencing Primers 1 & 2 (Illumina Inc., San Diego, CA, USA) and 10 µL of above purified DNA. PCR conditions were as follows: 95 °C for 1 min, 24 cycles of 95 °C for 30 s, 65 °C for 30 s, 72 °C for 30 s and a final extension step of 72 °C for 5 min. DNA quantitation and qualitative analysis of individual PCR products were performed on a MCE_-202 MultiNA with a DNA-1000 kit (Shimadzu, Kyoto). Samples were then pooled equimolar into groups of 95 samples (2 pooled libraries in total), with each pooled library being sequenced on a single Illumina HiSeq 2000 (Illumina, San Diego) lane by Macrogen (Seoul, Korea).

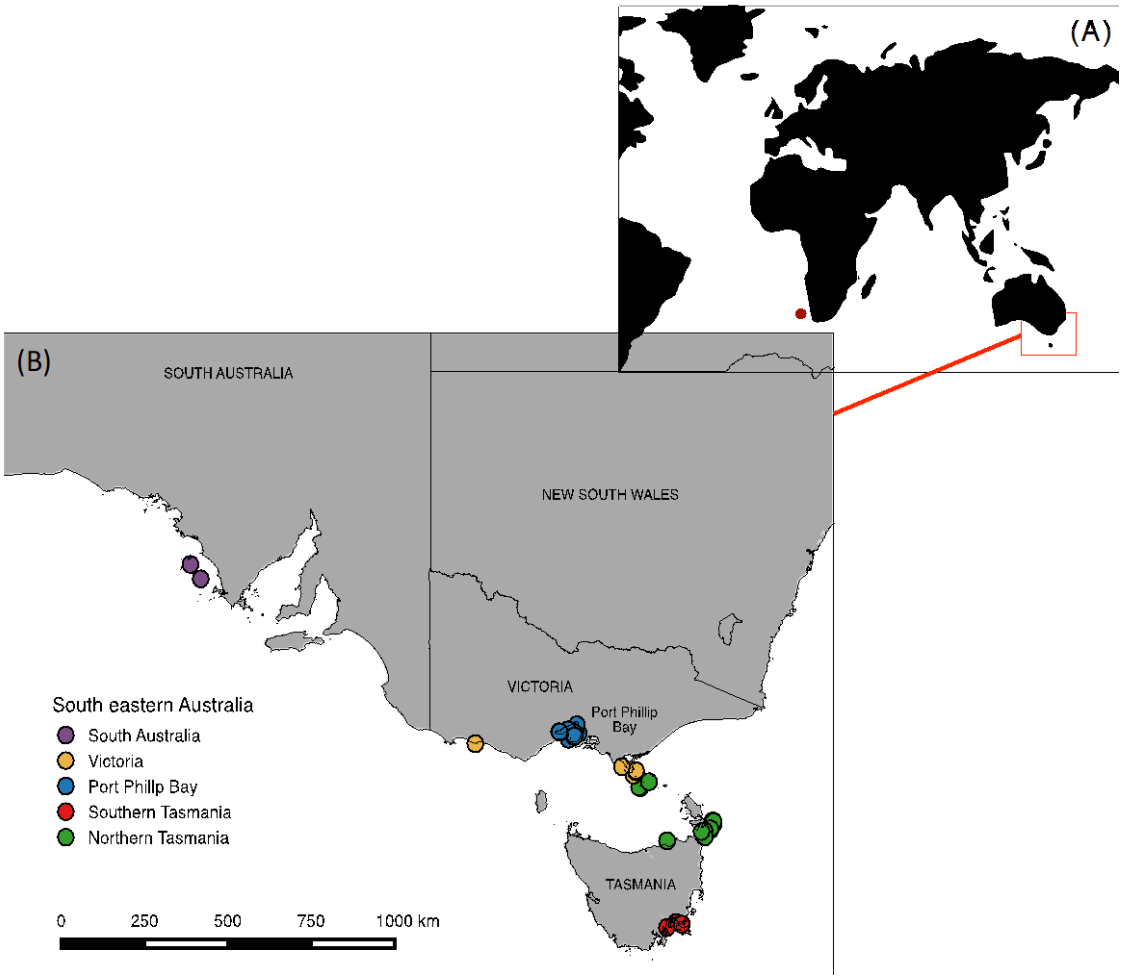

Figure 3.1 – Map of sampling locations for broadnose sevengill sharks. (A) Partial map of the world with sampling locations for Australia (N = 159) and South Africa (orange, N = 29). (B) Sampling for locations in south-eastern Australia, i.e. South Australia (SA, N= 4), Victoria (VIC, N= 9), Port Phillip Bay (PPB, N= 117), and Tasmania (northern, NTAS (N= 23) and southern, STAS (N= 6)). World map modified from data file downloaded from http://thematicmapping.org/downloads/world_borders.php

3.3.3 **Bioinformatics processing and genotyping**

Two samples were removed before filtering, one was an accidental replicate (ATAS_12921, from southern Tasmania), while the other sample had poor quality DNA that resulted in low sequencing depth (PPB_24, from Port Phillip Bay)**.** Low quality reads were initially removed

with Trimmomatic v.0.36 (AVGQUAL:20) (Bolger et al. 2014), followed by SNP identification from the remaining high-quality reads using the STACKS v.1.46 pipeline (Catchen et al. 2013). Within the pipeline, the *ustacks* program was used to create unique 'stacks' based on the requirements of a minimum coverage depth of 3 to create a stack and a maximum distance of 3 nucleotides allowed between stacks, while applying a chi-square significance level of 0.05 to call a heterozygote or homozygote SNP. The *cstacks* program was then run to catalogue these unique stacks, allowing at most 3 mismatches between sample tags, and ultimately generating a set of consensus loci. Subsequently, the *sstacks* program was then used to match stacks from each sample against the catalog. For analysis of the six populations, SNPs were filtered with the *populations* program based on the following criteria:

- at least 3 populations (=50%) must be present to process a locus
- at least 60% of individuals in a population is required in order to process a locus for that population
- a minimum minor allele frequency (MAF) of 0.05 is required to process a nucleotide site at a locus
- a minimum stack depth of 3 is required for individuals at a locus
- to retain only one random SNP per locus

After filtering, a total of 188 samples of *N. cepedianus* were analysed and a total of 2544 random single SNPs were retained. Australian samples totalled 159, from 5 locations within south-eastern Australia; South Australia (N=4), Victoria (N=9), Port Phillip Bay (N=117) and Tasmania (northern (N=23) and southern (N=6) (*Table 3.1*), with an out-group represented by South Africa (N=29).

3.3.4 **Genetic Analysis**

SNP frequencies were contrasted across all 6 sampled locations to determine patterns of population genetic structure within our dataset. A Tajima's D neutrality test was conducted

using MEGA 6 (Tamura et al. 2013). Observed and expected heterozygosities and inbreeding coefficients were calculated per population using GENODIVE 2.0b27 (Meirmans & Van Tienderen 2004). GENODIVE was also used to calculate genetic diversity such as pairwise F_{ST}. Initial analysis of population structure was conducted using a Principal Components Analysis (PCoA) and Discriminant Analysis of Principal Components (DAPC) was conducted using the R package *adegenet* (Jombart et al. 2010). The PCoA analysis was conducted for all locations and only the Australian locations, to eliminate any possible masking of differences by the out-group South Africa. The DAPC was conducted only on the Australian locations and the Bayesian information criterion (BIC) used to determine the number of genetic clusters within the data set. Structure plots were created using the program FastStructure (Raj et al. 2014). FastStructure analysis was run 100 times for each K from K2–6. To explore the relationships between the large number of individuals collected from the PPB site (n = 117), kinship coefficients of Loiselle et al. (1995) were calculated between individuals from PPB using GENODIVE 2.0b27 .

3.4 Results

3.4.1 Genetic diversity and population genetic structure

Genome scans of 188 *Notorynchus cepedianus* specimens from 6 locations distributed across south-eastern Australia and South Africa were performed by reduced genome representation sequencing. Illumina sequencing yielded a total of 2.6E+08 base paired reads, providing an average of 4E+06 base-paired reads per sample. *De novo* assembly using the STACKS bioinformatics pipeline yielded a total of 2544 SNPs. The Tajima's D neutrality test was -1.011361. Levels of diversity were consistent across sites with expected heterozygosities ranging from 0.3170 to 0.3395 (mean H_E = 0.3233), and observed heterozygosities ranging from 0.4957–0.5534 (mean H_O = 0.5141) (*Table 3.1*). Elevated observed heterozygosities and strong negative F_{IS} estimates for each sampling location (F_{IS} mean = -0.5910; range -0.537– -0.6302) indicate an excess of heterozygotes and no evidence of inbreeding within the sampled populations.

Table 3.1 – Genetic diversity of *N. cepedianus* in south-eastern Australia and South Africa (SAFR). Australian locations included, Southern Tasmania (STAS), Port Phillip Bay (PPB), South Australia (SA), Northern Tasmania (NTAS), and Victoria (VIC). Number of samples (N), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}).

Area (Region)	Population	N	H_O	H_E	F_{IS}
Australia (Oceania)	STAS	6	0.496	0.317	-0.564
	PPB	117	0.553	0.34	-0.63
	SA	4	0.528	0.333	-0.589
	NTAS	23	0.526	0.33	-0.595
	VIC	9	0.522	0.33	-0.583
South Africa (South Atlantic)	SAFR	29	0.502	0.31	-0.618
	Mean	188	0.514	0.323	-0.591

Overall, we detected low but significant levels of genetic differentiation between sampling locations with a global F_{ST} value of 0.012 ($P < 0.001$). When the South African samples were excluded, the global F_{ST} value decreased by half to 0.006, but remained significant ($P = 0.017$), indicating some structuring among Australian populations. The levels of genetic differentiation among Australian populations were driven primarily by small but significant differences between Victorian samples (PPB and VIC) and south Tasmanian samples (STAS) (*Table 3.2*). We detected no significant differentiation between Victorian, South Australian and Northern Tasmanian samples (*Table 3.2*). All Australian populations were significantly genetically differentiated from the South African population. The Principal Coordinate Analysis (PCoA) and STRUCTURE analysis was consistent with patterns of genetic differentiation observed from the F_{ST} values. The PCoA plot showed Australian locations clustering closely together, with the South African samples clustering as a distinct separate group (*Fig 3.2 & 3.3*). The

results of the DAPC on only the Australian locations also indicated one genetically mixed population. The BIC graph showed that K=1 is the lowest BIC value and therefore the most likely K (*Fig 3.4*). The DAPC table, testing for K=2, showed that all populations from Australia, except for South Australia had individuals in both clusters (*Fig 3.4*). Similarly, the STRUCTURE analysis identified two main genetic clusters within the data set, with the greatest likelihood of K = 2, with the South African samples forming a distinct cluster from the Australian cluster. A STRUCTURE plot of K = 6 indicated no subsequent sub-structuring within the Australian samples (*Fig. 3.5*).

Table 3.2 – Pairwise F_{ST} according to Weir and Cockerham (1984). Locations included, Port Phillip Bay (PPB), Victoria (VIC), South Australia (SA), Northern Tasmania (NTAS), Southern Tasmania (STAS), and one South African population (SAFR). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	PPB	VIC	SA	NTAS	STAS	SAFR
PPB	-					
VIC	0.000	-				
SA	0.000	0.000	-			
NTAS	0.000	0.001	0.000	-		
STAS	0.006**	0.007*	0.004	0.003	-	
SAFR	0.049***	0.047***	0.052***	0.053***	0.069***	-

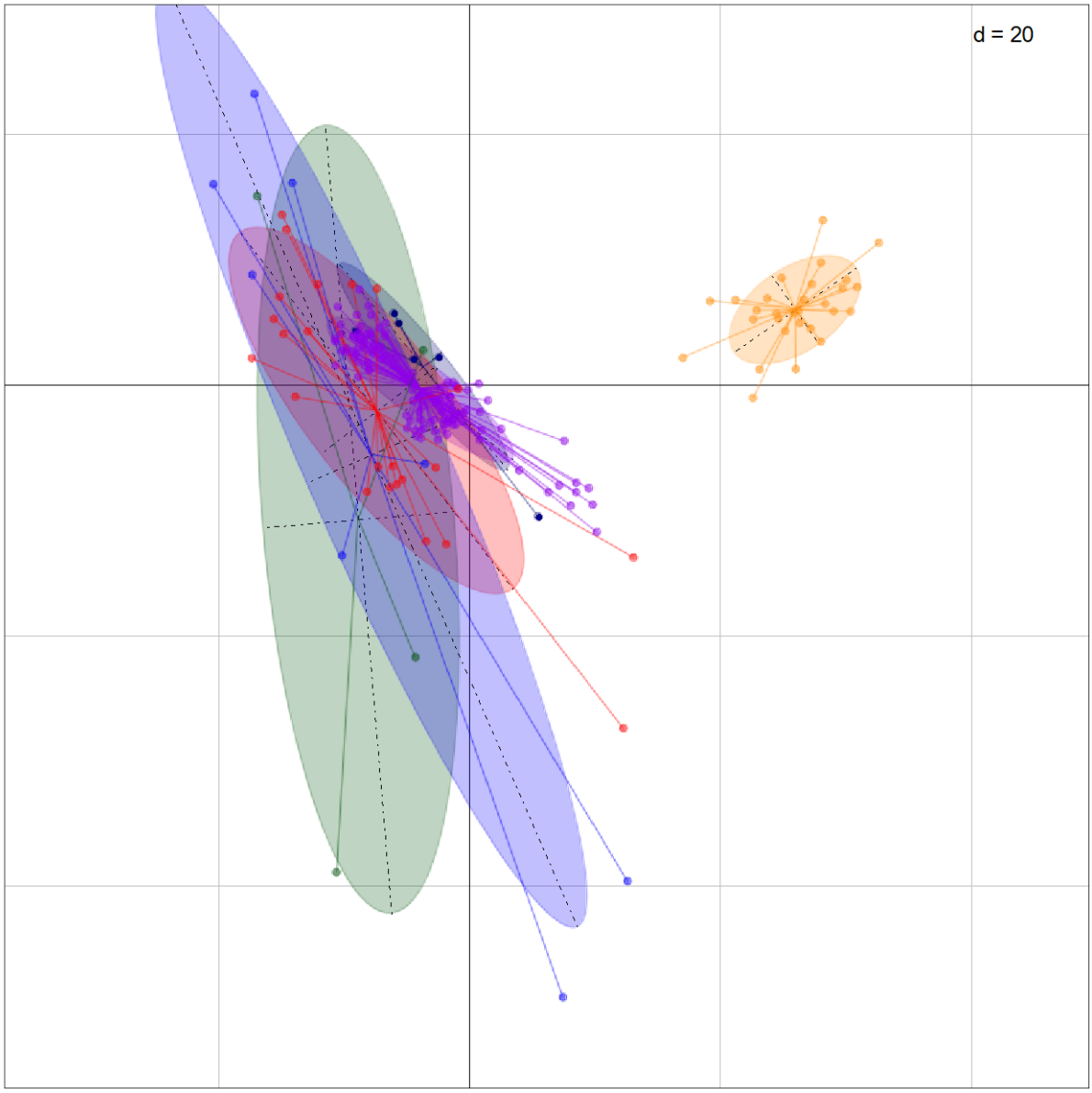

Figure 3.2 – Principal Components Analysis (PCA) of SNPs. Australian locations included, Southern Tasmania (dark blue), Port Phillip Bay (purple), South Australia (green), Northern Tasmania (red), Victoria (blue), and one South African population (orange).

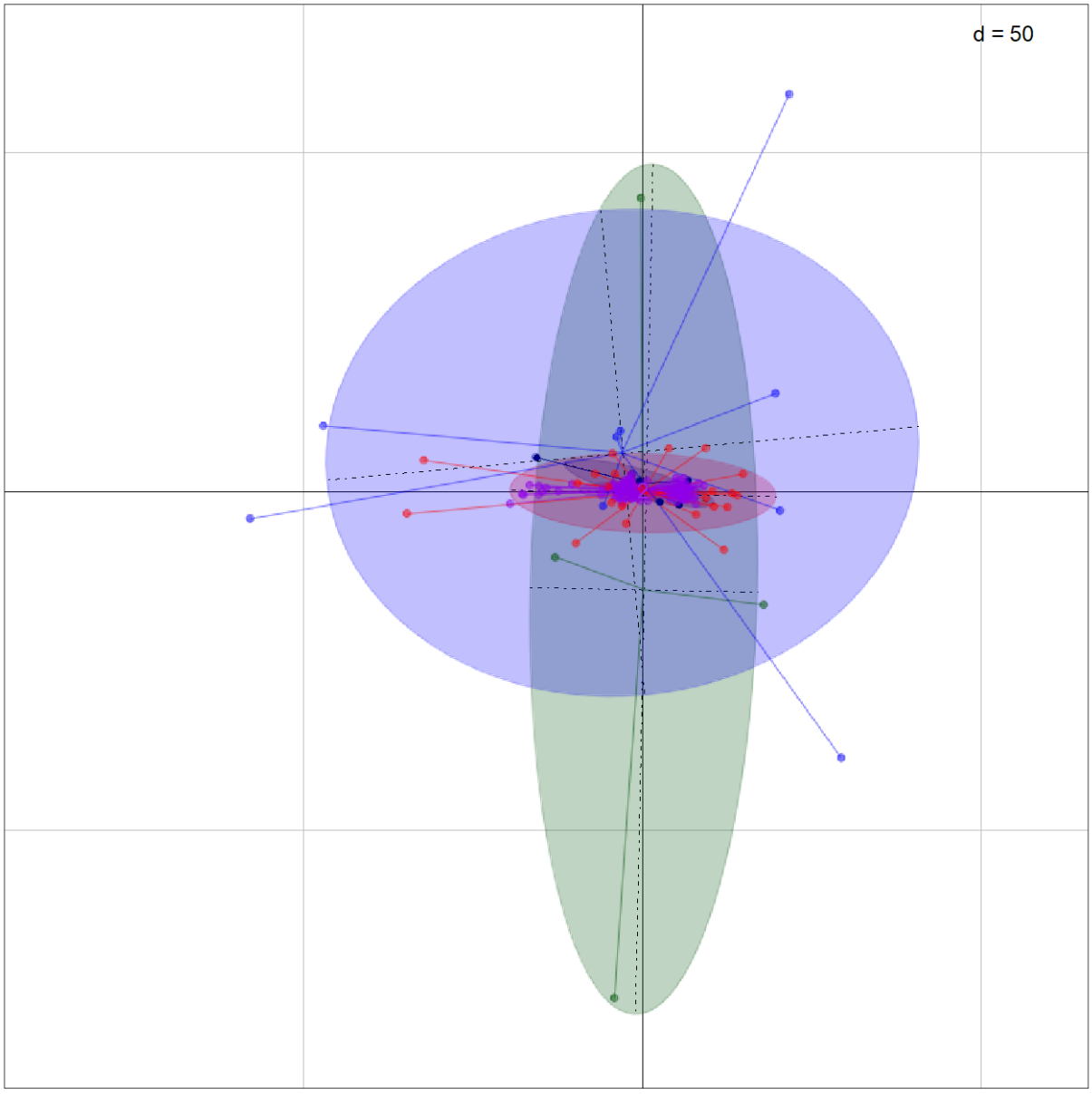

Figure 3.3 – Principal Components Analysis (PCA) of SNPs for Australian locations. Locations include Southern Tasmania (dark blue), Port Phillip Bay (purple), South Australia (green), Northern Tasmania (red), and Victoria (blue).

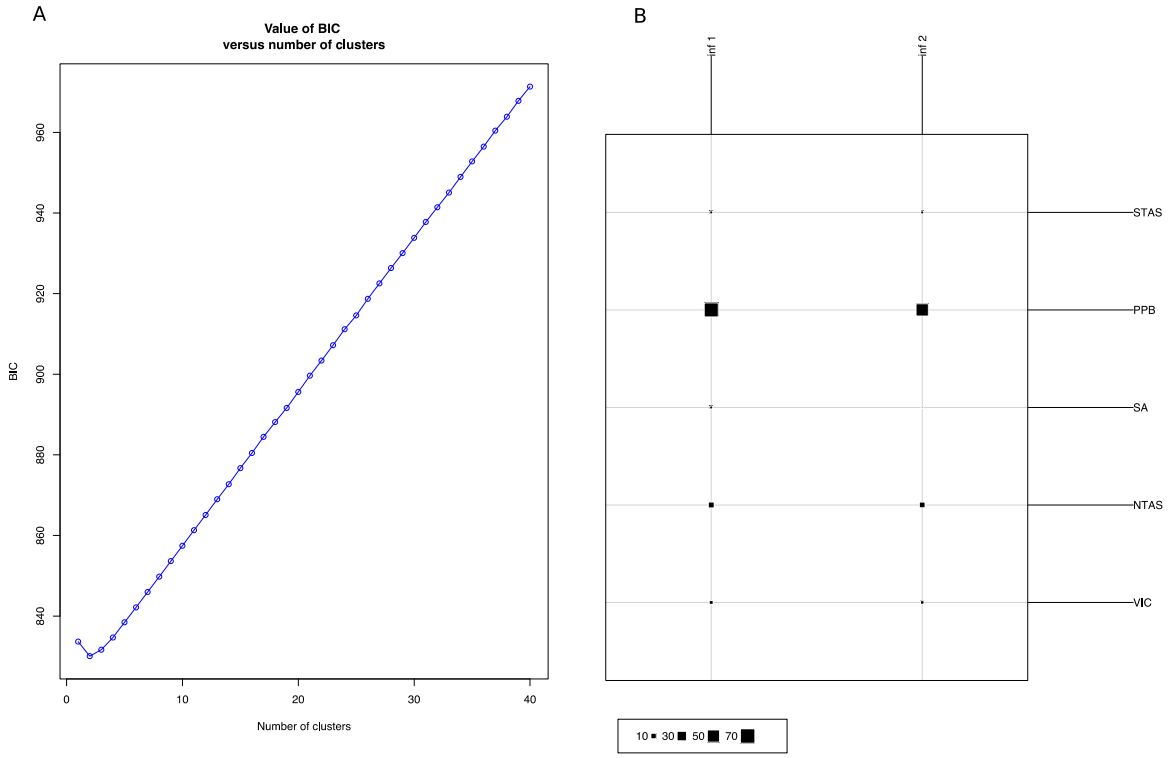

Figure 3.4 – A) BIC graph. B) Discriminant Analysis of Principal Components (DAPC) of SNPs. Australian locations included, Southern Tasmania (STAS), Port Phillip Bay (PPB), South Australia (SA), Northern Tasmania (NTAS), Victoria (VIC). The size of the squares represents the number of individuals from that location allocated to a particular genetic cluster.

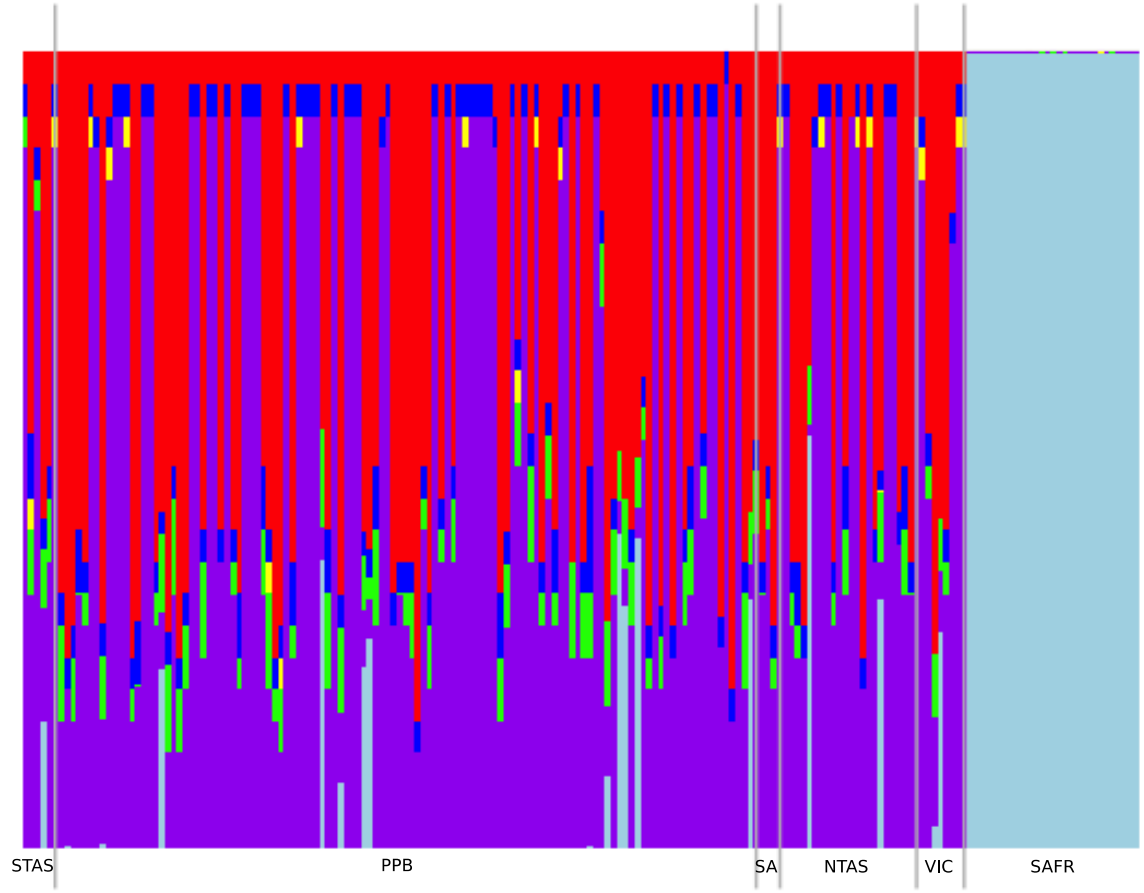

Figure 3.5 – Population Structure analysis with 2544 SNPs. Bar plots resulting from Structure analysis using all sampled locations – 5 Australian locations included, Southern Tasmania (STAS, red), Port Phillip Bay (PPB, blue), South Australia (SA, yellow), Northern Tasmania (NTAS, green), Victoria (VIC, purple), and one out-group population South African (SAFR, light blue). Model complexity that maximizes marginal likelihood = 2. Model components used to explain structure in data = 2.

3.5 Discussion

The primary aim of this study was to determine levels of genetic diversity, population structure and connectivity within *N. cepedianus* across south-eastern Australia. Our results showed similar levels of genetic diversity among populations, with only small levels of genetic differentiation between Victorian and southern Tasmanian populations. Additionally, there was

no evidence of inbreeding or that *N. cepedianus* have suffered declines that have impacted diversity estimates in Australia.

A clear distinction between all the Australian locations and the out-group South Africa was detected over all analyses, suggesting limited gene flow between these widely separated regions. This was consistent with findings presented in *Chapter 2*, which indicated clear differences between oceanic regions. The overall (Australia and out-group South Africa) average genetic diversity $H_O = 0.514$ and $H_E = 0.323$ and Australian (excluding South Africa) $H_O = 0.525$ and $H_E = 0.33$ found within *N. cepedianus* was similar to that observed along the western coast of the USA (Washington and California) and was generally lower than that of other shark species (Larson et al. 2015). Within Australia there was high connectivity among all sampled locations. The DAPC indicated a difference between SA and the other locations, however this was not reflected in the F_{ST} analysis, which indicated low significant differences between STAS, VIC and PPB samples (*Fig. 3.3*, *Table 3.2*). The PCA indicated no structuring for the Australian samples. Overall the results from the F_{ST}, PCoA and Structure are consistent with little genetic structuring among Australian subpopulations suggesting sufficient levels of dispersal and gene flow to prevent the accumulation of genetic differences between locations. This was different to results shown by Larson et al. (2015), which indicated two distinct populations between *N. cepedianus* sampled in coastal estuaries in California and Washington, with evidence of some mixing. This structure was suggested to be influenced by movement behaviour and seasonal site-fidelity of *N. cepedianus* to specific bays. *N. cepedianus* are known to seasonally migrate to coastal bays during the summer-fall months (Barnett et al. 2010c, Barnett et al. 2012, Williams et al. 2012). This has mainly been associated with feeding behaviour but in some areas such as Argentina, California and South Africa, there is some indication (presence of neonates) that this movement may also be associated with reproductive behaviour (Lucifora et al. 2005, Barnett et al. 2012, Awruch et al. 2014). Degrees of site fidelity to specific bays have also been observed with females tending to exhibit higher fidelity behaviour compared to males (Barnett et al. 2011, Williams et al. 2012, Ketchum et al. 2017). Philopatric behaviour towards a specific habitat may affect genetic structuring by reducing opportunities for genetic mixing between individuals from different areas through behavioural

and spatial separation. This has been shown for other species of sharks such as lemon shark (*Negaprion brevirostris*); sandbar shark (*Carcharhinus plumbeus*); blacktip shark (*Carcharhinus limbatus*) (Hueter et al. 2005, Keeney et al. 2005, Chapman et al. 2015). Natal philopatry or site-fidelity to nursery/pupping areas has not been observed for *N. cepedianus*, which may explain the connectivity between individuals from different locations in Australia. Similar findings were presented for scalloped hammerhead sharks, which also exhibited strong population structure between oceanic regions, but low genetic structuring within regions (Duncan et al. 2006). This indicates that *N. cepedianus*, similar to scalloped hammerhead sharks, show oceanic basin isolation of populations (see *Chapter 2*) over hundreds of thousands of years, while regional scale structure is influenced by movement and site-fidelity behaviour.

Results from the F_{ST}, PCoA, DAPC and Structure are consistent with little genetic structuring among Australian populations suggesting sufficient levels of dispersal and gene flow to prevent the accumulation of genetic differences between locations. We acknowledge that sample sizes for certain locations are low, however the use of SNP markers is advantageous as they can deal with this issue and have been shown to outperform microsatellites in detecting population structure (Larson et al. 2014). However, further studies are needed to determine if there is any hidden structuring within Australia. An in-depth investigation into the possible differences between VIC, STAS and SA is required, as there may be some evidence of restricted gene flow between these areas. Additionally, the inclusion of samples from areas such as NSW and Western Australia (WA) would strengthen knowledge about population structure and connectivity of *N. cepedianus* in Australia.

N. cepedianus are not considered to be a commercially important species but have been targeted in the past and remain a dominant by-catch species in the shark and finfish fishery (gillnet and long-line), as well as in recreational fishing (Barnett et al. 2012, De Wysiecki et al. 2018). Life history traits make this species susceptible to exploitation (Smith et al. 1999), however recorded landings of this species are sporadic or non existent. Reports of decreased abundance of *N. cepedianus* in Argentinian waters, in the south-west Atlantic, highlighted the need for the assessment of fishery-related mortality and conservation status of this species in the region (De

Wysiecki et al. 2018). This may be applicable to other regions where sevengill shark catch information is not frequently recorded.

Elucidating genetic structure and connectivity provides vital information on which populations or subpopulations maybe affected by fishery activity. For example, Larson et al. (2015) recommended that fisheries management and conservation policies for *N. cepedianus* along the U.S. west coast should be geographically population specific. Considering that this study suggests one well mixed population between the locations sampled within south-eastern Australia, it is recommended that any future management of *N. cepedianus* should not be confined to state boundaries but should be managed on a large scale as one population across their entire distribution within Australia.

3.6 Conclusion

Genomic data is an informative tool for ascertaining stock structure and connectivity within and between geographical populations. This information if vital for conservation and fisheries management as it can identify distinct management units, which can aid in focusing efforts towards populations at risk. This study showed that *N. cepedianus* sampled from VIC, SA, STAS, NTAS and PPB are highly genetically connected and exhibit low genetic structuring within this region. The vulnerability and conservation status of *N. cepedianus* in Australia is unclear. However, this species has been shown to be susceptible to fishing pressure (commercial and recreational) in other regions, where decreases in abundance have been observed. Thus, in order to determine the status and identify possible threats to this species, the establishment of data collection protocols is recommended to assess fishery-related impact on population stability. However, considering this species low commercial value, fisheries may not be the leading factor impacting this species and coastal degradation, habitat loss, pollution and climate change may have a greater effects on this species in Australia. Thus the protection of key coastal habitats such as breeding and feeding areas maybe key in maintaining healthy

stock populations. Additionally, management and conservation policies for this species should be incorporated across south-eastern Australia and managed as one stock across state boundaries, as population depletion in an area could affect the entire population. This can assist to ensure the long-term persistence of this ecologically important coastal apex-predator. Additionally, further research is necessary, particularly in the areas of key habitats such as nursery areas to fully understand drivers of population structure and connectivity.

4 Pupping area and neonate movement of broadnose sevengill sharks, *Notorynchus cepedianus*, in Port Phillip Bay, Australia

4.1 Abstract

The abundance of early life-stages strongly influences recruitment into the breeding stock and thus plays a crucial role in population structure, health and stability of species. Coastal areas and bays are commonly used as nursery and pupping areas by many shark species and are often important for neonate and juvenile shark development. However, the correct identification of these areas has been difficult, which has contributed to inefficient management and conservation of shark stocks. This study used acoustic telemetry to understand broadnose sevengill shark (*Notorynchus cepedianus*) neonate and juvenile movement patterns (site fidelity, seasonality and residency) within Port Phillip Bay (PPB) to determine its importance as a nursery or pupping area. Neonates (56 – 80 cm TL) were most abundant during mid-March to April. Residency within the bay for neonates was on average 12% (80 days) of their time at liberty over the >2-year period of the study (November 2014 – 29th January 2017). Neonates used less area than other life-stages within PPB, Kernel utilisation density, KUD_{95} = 76.22 km^2 and KUD_{95} = 128.99 km^2 respectively. However, there was a clear overlap of habitat usage, such as depth, between the different life-stages. Neonates were not recorded to return to the bay over the study period. Long-range movement indicated that sharks (both neonates and other life-stages) travelled to other state jurisdictional waters, such as Tasmania, New South Wales and coastal Victoria, reiterating the large-scale connectivity of sevengill shark populations in south-east Australia. Port Phillip Bay may be considered a pupping area for this species during the autumn – winter months, and is currently the only such area that has been documented for this species in south-eastern Australia.

This information is pertinent for a better understanding of sevengill shark ecology and for the establishment of management strategies for this species in southern Australia.

4.2 Introduction

An important aspect of understanding population structure and connectivity is identifying key habitats such as nursery areas. These areas provide a conducive environment for the growth and development of early life-stages. The protection of neonate and juveniles is important as they influence recruitment into the breeding population (Jackson et al. 2001, Heithaus 2007). This is particularly relevant to slow growing and late maturing species as a large breeding population is essential for replenishment and population stability (Kinney & Simpfendorfer 2009). Thus, these areas are important for the maintenance of the population structure and proliferation of the species. Though the importance of nursery habitats to the stock structure, connectivity and movement of species has been emphasised, a definitive delineation for what constitutes a nursery area has been ambiguous (Beck et al. 2001). Historically nursery areas were defined as, areas where young are birthed and/or reside as they develop (Heupel et al. 2007). This has led to regions being classified as nurseries solely on the presence of neonates and/or young-of-the-year (YOY) (Castro 1993, Simpfendorfer & Milward 1993, Blackburn et al. 2007). Heupel et al. (2007) proposed a more comprehensive definition of a shark nursery based on three criteria: *1)* abundance (higher than in other areas), *2)* residency (remain or return to area for extended periods) and *3)* inter-annual (return to area across years) use of areas by neonates or YOY. Heupel et al. proposed other terminologies, such as pupping/birthing areas, to define areas where neonates and YOY are present but do not meet the above-mentioned criteria. The identification and classification of critical habitats, is required to understand habitat use of a species throughout its lifecycle.

Shark research has shown a strong habitat and taxonomic bias towards tropical habitats and species and more work needs be directed towards temperate, deep-water and

pelagic habitats and associated shark species to fully understand the use and benefits of these areas for shark stocks (Heupel et al. 2018).

Many shark species are known to use coastal bays as nursery/pupping areas, (Heupel et al. 2007, Speed et al. 2010, Heupel et al. 2018). Site fidelity and movement between nursery and other habitat areas directly affect connectivity between populations, genetic divergence among regions, and overall population dynamics of a species (Keeney et al. 2005). Insight into the movement between and use of nursery areas is crucial for understanding population dynamics, as it can connect or isolated populations using these areas. Focus has often been placed on the movement of mature life-stages (Braccini et al. 2017). However, it is also important to understand early life-stage movement and behavior, as this is essential to identifying nursery areas, understanding spatio-temporal usage patterns, as well as connectivity to adult populations.

Sevengill shark, *Notorynchus cepedianus* are commonly found in temperate coastal bays and estuaries worldwide (Compagno et al. 2005, Barnett et al. 2012). In Australia, this species is common in coastal areas and estuaries around Tasmania, South Australia, Victoria and New South Wales (Last & Stevens 2009). They are one of the most abundant predators in shallow coastal areas, especially during the summer months (Ebert 1989, Lucifora et al. 2005, Last & Stevens 2009, Barnett et al. 2010d, Barnett et al. 2011). Ecologically, *N. cepedianus* exhibit similar trophic importance as other large shark species such as tiger sharks and white sharks (Cortés 1999, Barnett et al. 2012). Studies on this species have mainly focused on adult and sub-adults (Barnett et al. 2012 and references within). Therefore, gaps in ecological knowledge persist in respect to early life stages, including the identification of critical habitats such as nursery/pupping areas. Long distance movement of adult *N. cepedianus* between coastal bays has been recorded. Along the west coast of the US, movement of *N. cepedianus* were recorded between coastal bays in California, Oregon and Washington states (Williams et al. 2012, Ketchum et al. 2017). Similar coastal movement has also been shown for this species in Australia with individuals moving from south-eastern Tasmania to New South Wales and the Bass Strait (Barnett et al. 2011, (Stehfest et al. 2014). In Washington (Williams et al. 2011) and Tasmania (Barnett et al. 2010c, Barnett et al. 2012, Barnett & Semmens 2012) this movement has been associated with feeding

behaviour, whereas in California (Ebert 1989, 2003) and Patagonia (Lucifora et al. 2005) seasonal abundance of *N. cepedianus* in coastal bays has been suggested to be related to reproductive activity (nursery/pupping). Specific studies on neonate or juvenile movement have not been conducted for this species and are required to accurately identify key habitats such as nursery and pupping areas.

Anecdotal accounts by fisherman have indicated neonate presence in Port Phillip Bay, Victoria and areas near coastal South Australia (*Fig. 4.1*). Port Phillip Bay (PPB) is the largest coastal bay in Victoria, Australia and is a nursery and spawning area for shark and fish species such as the school sharks (*Galeorhinus galeus*) (Olsen 1954, Stevens & West 1997), and snapper (*Chrysophrys auratus*) (Hamer et al. 2011, Hamer & Conron 2016). To ascertain if Port Phillip Bay is a nursery/pupping ground for *N. cepedianus* in south-eastern Australia, this study used Catch per unit effort (CPUE) to determine the abundance and occurrence of neonate and juvenile *N. cepedianus* and acoustic telemetry coupled with mark recapture to determine residency and inter-annual return (site-fidelity) within Port Philip Bay. Movement patterns of adults and juveniles within the bay were also investigated to understand movement patterns, juvenile behaviour and identify areas of high density. Overall this study contributes to a wider understanding of population structure, connectivity and dynamics of *N. cepedianus* and provides information for the development of management strategies by identifying an important nursery/pupping ground in south-east Australia.

4.3 Methods

4.3.1 **Study Site and Acoustic Receivers**

4.3.1.1 ***Location***

Port Phillip Bay (PPB) is located in Victoria, south-eastern Australia, adjacent to the city of Melbourne. It is the largest sheltered bay (approx. 1,930km^2) in Victoria. The bay is separated from the Bass Strait by the Bellarine and Mornington Peninsulas, which form a narrow entrance approximately 3 km wide (*Fig. 4.1*). The deepest area is

24m with about half the bay less than 8m in depth. The Yarra/Maribyrnong is the major river that discharges into the northern area of the bay. There is a long residence time of water within the bay (12 – 16 months), as a result of the bay's narrow entrance which partially isolates the inner bay basin from the ocean water outside the bay (Harris et al. 1996). Water temperatures vary between approximately 9 – 11°C in winter and 23 – 24°C in summer. Typically salinity values are similar to ocean values outside the bay, between 35 – 36 ppt, however periods of hyposaline and hypersaline can occur due to shifts in evaporation and rainfall (Lee et al. 2012).

Port Phillip Bay is an area of high productivity and supports an array of marine life, such as marine mammals (Australian fur seals, dolphins and whales), chondrichtyans (sharks, rays, elephantfish), teleosts (e.g. snapper, trevally, Australian salmon, flatheads, flounders, whiting) and invertebrates (Harris et al. 1996). The bay is also a nursery/spawning area for a variety of commercially important species such as school shark (*Galeorhinus galeus*) and snapper (*Chrysophrys auratus*) (Walker 1998) . Commercial (gillnet and longline) and recreational fishing practices occur within the bay and the port of Melbourne is one of the busiest in Australia.

4.3.1.2 ***Receiver array and mooring***

An array of 46 acoustic receivers comprised of VR2 and VR2W models (VEMCO Ltd, Halifax, Canada), were deployed in PPB. Receivers were active from November 2014 – 29 January 2017 to provide approximately two years of tracking information. Both models could detect all transmitters. Nine receivers were arranged in an overlapping range curtain across the entrance of the bay (The heads) to detect sharks leaving and entering the bay, the remaining receivers were spread across the bay (*Fig 4.1*). The heads receivers were spaced at approximately 600m intervals so that there was some overlap in detection ranges of receivers (Hamer & Mills 2017). Details of receiver coordinates, and data are included in *Appendix 7.1*.

Two receiver mooring methods were used; 1) a concrete filled car tyre with two PVC pipes and star pickets embedded in the centre, the receivers were fixed to the highest point on the tallest star picket with cable ties (main method); and 2) a concrete filled car tyre with a chain attachment to which a rope and two floats were attached, the

receiver was mounted onto the rope between the two floats. This approach was used for deeper sites as it could be deployed directly from the vessel without diver assistance (Hamer & Mills 2017).

4.3.2 **Shark collection and tagging**

N. cepedianus were captured using bottom-set baited longlines and rod and reel from November 2014 – April 2015 (summer – autumn), after the receivers were deployed in Port Phillip Bay. Fishing occurred between 12am – 10am for 6 – 10 hours with soaking times of approximately 1½ - 2 hours per set. The majority of fishing occurred from one commercial snapper fishing vessel, which used approximately 200 small circle hooks (Mustad Hooks 8260D 5/0) per longline set with 2 – 4 sets per trip. Additional fishing occurred using a research vessel, with 50 hooks per set (100 small (Mustad Hooks 8260D 5/0) and 100 large (Mustad Hook 39960D 14/0) circle hooks) and 4 – 8 sets per trip.

Life-stages were categorised according to these size classes; neonates <80 cm total length and other life-stages; juveniles >80 – 140, sub-adult >140 – 190 (male) and >140 – 209 (female), adult >190 (male) and >209 cm total length (female) (Awruch et al. 2014). Sixty-one transmitters were surgically implanted into the abdomen of 43 neonates and 18 other life-stage *N. cepedianus* (*Appendix 7.2*) (see tagging procedure below). Transmitters comprised of the following *VEMCO* tag types, nine high powered V13s (neonates), six low powered V13 Pressure tags, which record depth (4 neonates, 2 other life-stages), thirty V13 low powered (30 neonates), and sixteen low powered V16 tags (used for larger sharks >125 cm, other life-stages) (*Appendix 7.2*). Tags had a battery life span between 428 – 3650 days (*Appendix 7.2*). Range testing according to Hamer and Mills (2017) indicated a transmitter detection range of approximately 400m for all tags. Sharks were also fitted with fin tags comprised of *DROVER* Rototags (used for larger sharks, >125 cm TL), *HALLPRINT* PDAT plastic dart tags, and small fish dart tags (used for neonate and juvenile sharks).

4.3.3 **Fin and Acoustic tagging procedure**

Once captured, sharks were either brought on board or secured alongside the boat, dependent on size. Sharks were measured from the snout to tip of the tail to the nearest cm (Total length, TL), sex recorded and a small tissue samples were obtained for genetic analysis (fin clip). Small sharks (<100 cm) were brought onboard and kept in a large commercial fishing icebox filled with seawater, before and after surgery to assist in recovery. Surgeries for small sharks were performed on a foam mattress. When large sharks were brought onboard a hose with running seawater was pumped through their mouth over their gills during surgery. Large sharks secured alongside the boat were maneuvered into an inverted position to perform surgeries.

Surgical procedure followed that of Barnett et al. (2011). Surgical instruments and tags were kept in antiseptic solution (Betadine, 250ppm), then rinsed with sterile distilled water bath and diluted Betadine solution before use. Before incisions (1-2cm (small sharks) and 2-3cm (large sharks) into the abdominal cavity were made, the area was cleaned with a diluted Betadine solution and 0.1-2ml of local anesthetic (Lignocaine) was injected into the muscle tissue at the center of the incision site. The transmitter was inserted and the incision was closed with medical sutures. Once the surgery was completed a fin or dart tag was attached on or near the dorsal fin before release. Overall the entire tagging procedure was accomplished in 3-5 minutes. All sharks swam away strongly once released back into the water.

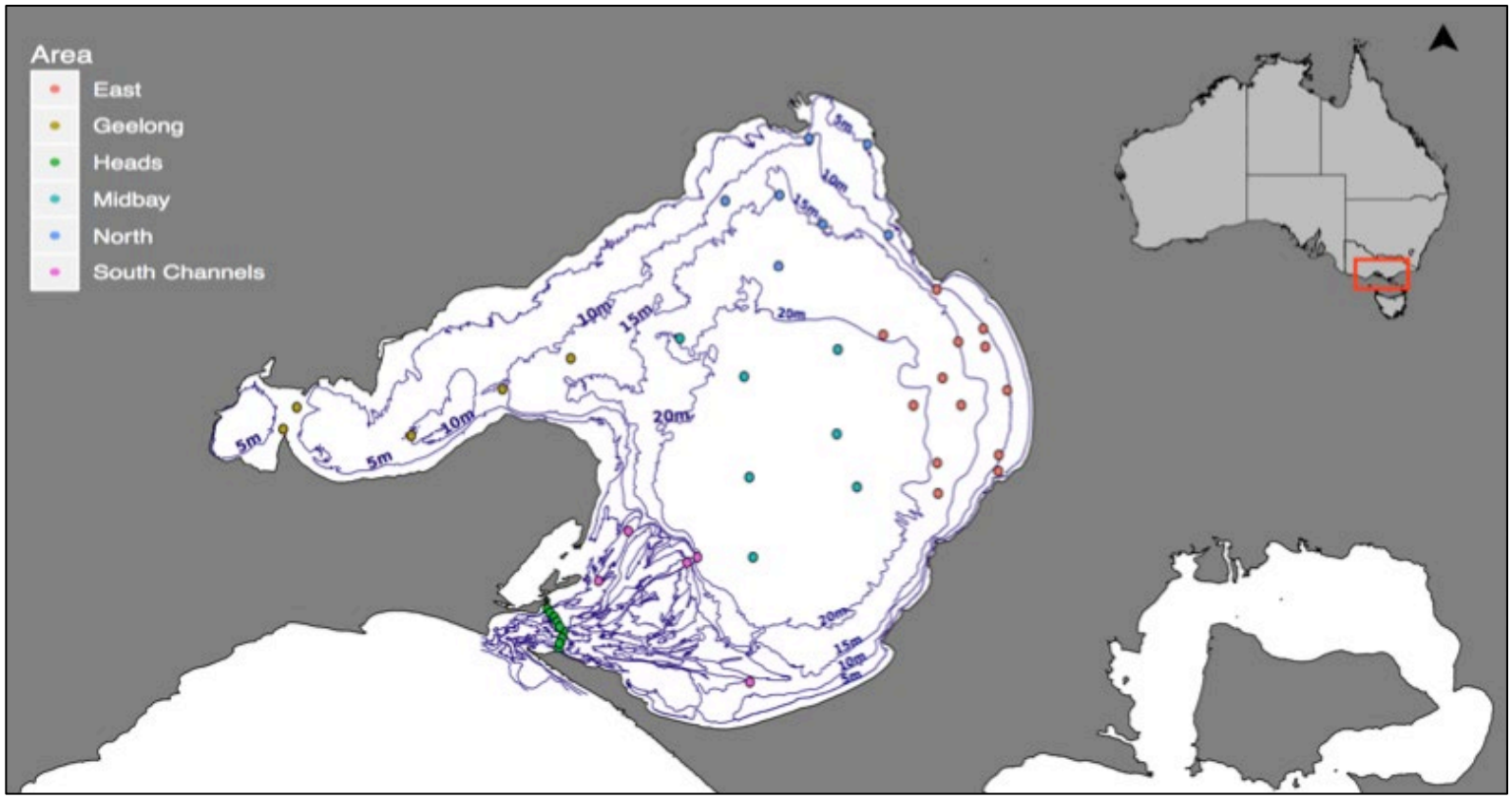

Figure 4.1 – Map of study site, receiver location and bathymetric chart within Port Phillip Bay, Victoria

4.3.4 **Data Analysis**

4.3.4.1 ***Abundance***

Catch per unit effort (CPUE) from long-line fishing, was used to estimate relative abundance of neonate *N. cepedianus* in PPB. This was defined as the number of sharks captured per 50 hooks (using bottom-set longline fishing methods) per hour soak time.

4.3.4.2 ***Seasonal residency***

To determine the seasonal residency within PPB by neonates and other life-stages, the total number of days an animal was detected in the bay was divided by the total number of days that animal had been at liberty since tagging (date tagged to end of study period, 29/01/2017). A shark was considered present in the bay on a given day if it was detected by any of the receivers within the bay. Sharks were considered to have departed the bay if they were detected by the entrance receiver (Heads) and undetected by any receiver within the bay for more than 24 hours.

4.3.4.3 ***Movement patterns and habitat usage***

Spatial utilisation of the PPB by neonates and other life-stages was determined by examining the total number of hours each shark was detected at the geographical location of stand-alone receivers (*Fig 4.1*) or groups of receivers (East, Geelong, Heads, Midbay, North and South channel) in a given day. If a shark was detected at least once in a given hour for that day then it was considered as being present during that hour. Using the stand-alone and grouped receivers, spatial overlap between neonates and other life-stages was compared using niche overlap analysis in the *EcoSimR* package in R (Gotelli et al. 2015). The Pianka's index (O) was used and permutated 1000 times using the RA3 algorithm (Meyer et al. 2009). The degree of overlap is presented in a 0–1 scale, where 0 means no overlap and 1 means complete overlap. Brownian bridge kernel utilisation density (KUDs) was used to estimate preference for areas around each receiver and approximate the area used by neonates and other life-stages. The 50% fixed kernel indicate the receivers most often used and the 95% indicate the overall use of available receivers by sharks. KUDs were estimated using the R packages

adeHabatitHR, adeHabatitLT, and *adeHabatitMA* (Calenge 2006). Individual receivers in PPB were assigned a depth category, <5-10 m (13), 10-15 m (14), 15-20 m (13), and >20 m (6), based on the average depth covered by the receiver range (*Appendix 7.1*) to calculate selection (w_i) and overlap in use by neonates and other life-stages for a particular depth. Receivers were also grouped into regions, such as East, Geelong, Midbay, North, South Channel and Heads (entrance curtain receiver) to examine broader scale residency patterns within the bay. A log-likelihood (χ^2) statistic was used to test for individual habitat selection (w_i) for particular depths and areas with associated confidence intervals (Manly et al. 2003). Analyses were conducted using the *adeHabatitHS* package in the R using Manly's selection ratio for habitat selection design III (Calenge 2006). Selection ratios >1 indicate a preference, whereas values <1 indicate avoidance of a particular receiver (Manly et al. 2003). Circular statistics were used to determine the diel use of each depth category by neonate and other life-stages in Port Phillip Bay using *ggplot2* in R.

4.3.4.4 ***Long-distance movement patterns***

Acoustic tags were registered with the Integrated Marine Observing System (IMOS) (IMOS 2018), animal tracking facility, which has acoustic receivers along the Australian coastline (*Fig. 4.8*). Each fin tag had contact information for reporting recaptures. Movement and distance travelled were calculated using the R package " *VTrack*" (Campbell et al. 2012). All recaptures were mapped to illustrate long distance movement patterns of *N. cepedianus* outside of Port Phillip Bay.

4.4 Results

4.4.1 **Abundance**

A total of 108 sharks were caught using both longline (100) and rod-reel (8) fishing methods. There was close to a 50:50 ratio of male to females caught, 46% and 54% respectively. Sharks categorised as neonate comprised 73% (79) of the catch with 27%

composing other life-stages (7% (7) juveniles, 10% (11) sub-adults and 10% (11) adults). The majority of neonates were caught mid-March to April, while adults were caught sporadically throughout the tagging season.

Over 26 fishing trips 44 longline sets were conducted; seven sets of 50 large circle hooks and 37 sets of small circle hooks (25 sets of 200 and 12 sets of 50 small hooks). The total CPUE from longlining was 0.20 sharks per 50 hook/hr. Longlining with small hooks contributed to approximately 84% of all fishing effort. The CPUE for longlining with small hooks was 0.2 and 0.03 per 50 hook/hr for neonates and other life-stages respectively (*Fig. 4.2*). The majority of fishing effort used small hooks and thus predominantly targeted smaller animals though larger animals were also caught. Peak catch rates of neonates were approximately 2 sharks per 50 hook/hr in late March.

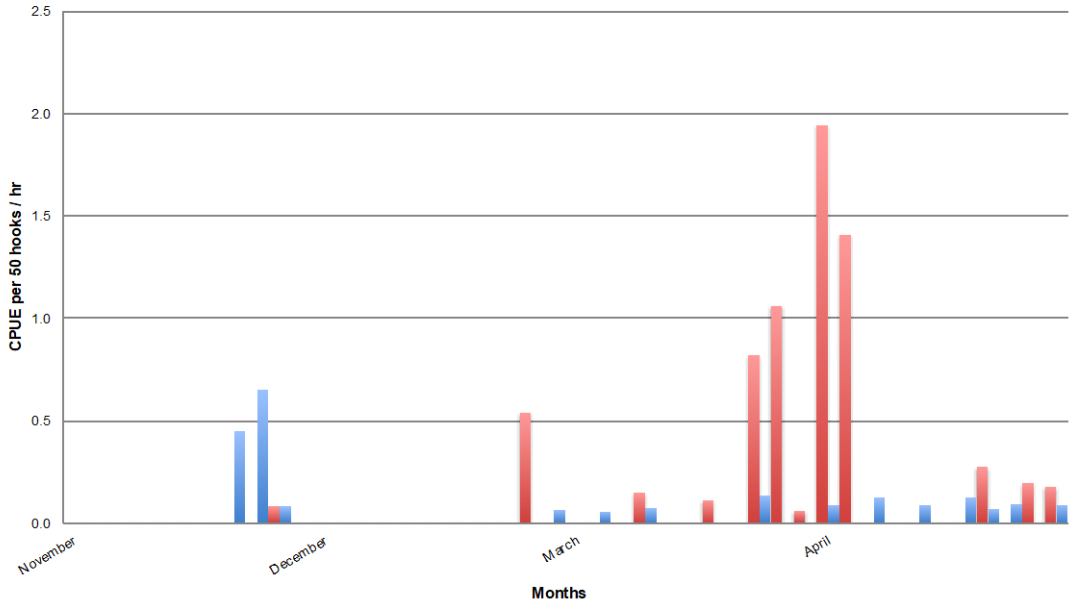

Figure 4.2 – Histogram of total CPUE per 50 hooks per hour effort for *N. cepedianus* caught from November 2014 – April 2015 in PPB. *Blue* represents other life-stages and *Red* represent neonates

4.4.2 **Residency and site-fidelity**

A total of 59 out of the 61 *N. cepedianus* acoustically tagged from November 2014 to April 2015 were detected in PPB at some point from November 2014 – 29 January 2017. Size range of tagged sharks was between 56 – 260 cm TL, with females and males representing 57% and 43% respectively of acoustically tagged sharks. Fifty-nine of these sharks, comprising 42 neonates and 17 other life-stages (2 juvenile, 7 sub-adults, and 8 adults) were detected within the bay (*Table 4.1, Fig. 4.3*). Overall, 12 (29%) neonates were detected leaving PPB (June-July 2015), and did not return to the bay within the study period. Twenty-seven (64%) neonates were detected within the bay after tagging but where not detected leaving the bay and remained undetected within the bay after June-July 2015. However, three of these neonates were recaptured outside of the bay despite not being detected leaving the bay by the entrance receivers. Sharks may have been able to pass through the detection range of the entrance receivers while the tag was on nominal delay, the tag transmissions may have been masked by reef habitat in the entrance region if sharks moved close to the seabed or by rough weather conditions (Hamer & Mills 2017). There were also three (7%) neonates that resided in the bay for extended periods (9 month to > 1 year) of time before detections ceased. Shark SG15 (110 cm TL, other lifestage) was only detected once after tagging, the day after it was tagged, however she was recaptured by a fisher within the bay 17 days after tagging and was not released. Three other sharks were detected < 2 days within the bay, sharks; SG18 (62 cm TL, neonate, F) was detected once 47 days after tagging; SG94 (213 cm TL, adult, M) was detected twice 10 days after tagging and SG39 (75.5 cm TL, neonate, F) was detected 3 days after tagging. Sharks from other life-stages exhibited more residency, six sharks (SG3, 4, 20, 69 92 and 95) stayed in the bay for the study period, three sharks (SG30, SG 90, SG93) left the bay (July-August 2015, 77 – 95 day after tagging) and did not return, and six sharks (SG1, 2, 5, 87, 91 and 104) left and returned to the bay at least once during the study period. On average, neonates were present in PPB for 80 days (range 3 – 469 days) representing approximately 12% of their time at liberty (*Table 4.1, Fig. 4.3*). The three neonates that remained in PPB the longest were SG49 (469 days), SG29 (469 days) and SG41 (270 days). The average days present in PPB for other life-stages was 413 days (range 1 – 783 days), which represented 59% of their time at liberty.

Figure 4.3 – Abacus plot showing detection dates for all *N. cepedianus* acoustically tagged in Port Phillip Bay between November 2014 – January 2017. N refers to neonates tagged. Each line represents an individual shark. Blue dots represent detection at the entrance receiver ('The Heads'), red dots represent all other receiver detections

Table 4.1 – Details of *N. cepedianus* fitted with acoustic tags in Port Phillip Bay. Tagging date is the date animal was tagged. Total length in cm is TL. Last detection day is the last date the tag was detect by the PPB receivers. Days detected are the total number of days a shark was detected by the Port Phillip Bay acoustic array. Time at liberty is the total number of days between the date a shark was tagged and the end of the study period (29-Jan-2017). % of days detected represents the percentage of days detected from the date tagged until the end of the study 29-Jan-2017. The symbols in the Return column indicate whether a shark remained in PPB (+), departed PPB and did not return (×), departed and returned to PPB (∞), was detected by AATAMS receivers outside PPB (*), were recaptured by fishers inside (<) or outside (>) PPB.

						Acoustic Tag					
Area tagged	**Shark ID**	**Date Tagged**	**Sex**	**Life stage**	**TL (cm)**	**Last detection day**	**Days detected**	**Time at liberty**	**% of days detected**	**Recapture date**	**Returns**
Geelong Arm	SG3	28-Nov-14	F	Sub-Adult	190	19-Jan-17	783	793	99	3-Sep-15	*
	SG4	28-Nov-14	F	Adult	240	13-Dec-16	746	793	94		+
	SG5	28-Nov-14	F	Sub-Adult	206	19-Sep-15	295	793	37		∞
	SG15	12-Mar-15	F	Juvenile	110	13-Mar-15	1	689	0.1	30-Mar-15	<
	SG19	25-Mar-15	F	Neonate	65	6-Jun-15	73	676	11		×
	SG48	31-Mar-15	M	Neonate	73	21-May-15	51	670	8		
				Average TL = 147.33							
Mid-Bay	SG8	2-Mar-15	F	Neonate	62	31-May-15	90	699	13		x
	SG9	2-Mar-15	F	Neonate	60	25-Jun-15	115	699	16	8-Mar-16	x, >
	SG10	2-Mar-15	F	Neonate	72	24-Apr-15	53	699	8	28-Mar-15	<
	SG11	2-Mar-15	F	Neonate	59	25-Apr-15	54	699	8		
	SG17	14-Mar-15	M	Neonate	58	7-Jun-15	85	687	12		×
	SG18	14-Mar-15	F	Neonate	62	30-Apr-15	47	687	7		

SG20	29-Mar-15	F	Sub-Adult	200	8-Jan-17	651	672	97		+
SG21	29-Mar-15	F	Neonate	64.5	28-Apr-15	30	672	4		
SG22	29-Mar-15	F	Neonate	72.5	20-May-15	52	672	8		x
SG23	29-Mar-15	M	Neonate	74	3-May-15	35	672	5		
SG24	29-Mar-15	F	Neonate	78	28-Apr-15	30	672	4		
SG25	29-Mar-15	F	Neonate	70.5	6-May-15	38	672	6		
SG26	29-Mar-15	M	Neonate	76	31-May-15	63	672	9		x
SG27	29-Mar-15	M	Neonate	67	23-May-15	55	672	8		
SG28	29-Mar-15	F	Neonate	73	23-Aug-15	147	672	22		
SG29	29-Mar-15	F	Neonate	60.5	10-Jul-16	469	672	70		x
SG30	29-Mar-15	M	Adult	210	2-Jul-15	95	672	14		x
SG31	29-Mar-15	F	Neonate	64	28-May-15	60	672	9		
SG32	29-Mar-15	F	Neonate	61.5	20-Jul-15	113	672	17	3-Dec-16	x, >
SG33	29-Mar-15	M	Neonate	70	18-May-15	50	672	7		
SG49	10-Apr-15	M	Neonate	73	27-May-15	47	660	7		x
SG50	10-Apr-15	M	Neonate	71.5	14-May-15	34	660	5	11-Jan-16	x, >
SG51	10-Apr-15	M	Neonate	79	24-May-15	44	660	7		
SG52	10-Apr-15	F	Neonate	56	1-Aug-15	113	660	17		
SG53	10-Apr-15	F	Neonate	77.5	1-Jun-15	52	660	8		x
SG54	10-Apr-15	F	Neonate	71	9-May-15	29	660	4	17-Aug-15	*
SG55	10-Apr-15	F	Neonate	62	13-Jun-15	64	660	10		x
SG56	10-Apr-15	M	Neonate	67	27-May-15	47	660	7		
SG57	10-Apr-15	M	Neonate	72	14-May-15	34	660	5		

	SG69	10-Apr-15	F	Sub-Adult	180	29-Jan-17	660	660	100		
	SG87	12-Apr-15	F	Sub-Adult	196	20-Nov-16	588	658	89		∞
	SG92	22-Apr-15	F	Adult	215	7-Dec-16	595	648	92		
	SG93	22-Apr-15	F	Juvenile	125	21-Aug-15	121	648	19	9-May-16	x, *
	SG94	22-Apr-15	M	Adult	213	2-May-15	10	648	2		
	SG95	22-Apr-15	F	Sub-Adult	185	10-Jan-17	629	648	97	3-Apr-18	<
	SG104	25-Apr-15	M	Adult	225	15-Dec-16	600	645	93		∞
				Average TL = 99.51							
Mornington	SG34	30-Mar-15	M	Neonate	67	6-Jun-15	68	671	10		
	SG35	30-Mar-15	M	Neonate	71	10-Jun-15	72	671	11		x
	SG36	30-Mar-15	M	Neonate	70	*Not detected*	0	671	0		
	SG37	30-Mar-15	M	Neonate	60	22-May-15	53	671	8		
	SG38	30-Mar-15	M	Neonate	72	11-May-15	42	671	6		x
	SG39	30-Mar-15	F	Neonate	75.5	2-Apr-15	3	671	0.4		
	SG40	30-Mar-15	M	Neonate	75.5	27-Jun-15	89	671	13		
	SG41	30-Mar-15	M	Neonate	71.5	25-Dec-15	270	671	40		
	SG42	30-Mar-15	F	Neonate	70.1	9-Jul-15	101	671	15		
	SG43	30-Mar-15	F	Neonate	60	9-Apr-15	10	671	1		
	SG44	30-Mar-15	M	Neonate	70.5	12-Apr-15	13	671	2		
	SG45	30-Mar-15	M	Neonate	72	6-May-15	37	671	6		x
	SG46	30-Mar-15	F	Neonate	63.5	11-Jul-16	469	671	70		∞
	SG47	30-Mar-15	M	Neonate	61.5	19-May-15	50	671	7		

					Average TL = 68.58						
South Channel	SG1	26-Nov-14	F	Adult	248	15-Nov-15	354	795	45		∞
	SG2	26-Nov-14	F	Sub-Adult	184	12-Sep-16	656	795	83	23-Sep-15	∞,*
	SG13	3-Mar-15	F	Adult	260	*Not detected*	0	698	0		
	SG90	17-Apr-15	M	Adult	217	3-Jul-15	77	653	12		x
	SG91	17-Apr-15	M	Adult	218	10-Nov-16	573	653	88		∞
					Average TL = 225.40						
Overall Average TL = 107.44											

4.4.3 **Movement and spatial overlap within PPB**

Overall, other life-stages were detected at more receivers within the bay than neonates. A comparison of neonate and other life-stage sharks showed a significant overlap in the use of receivers in PPB (*O* = 0.7, *p < 0.001*) (*Fig. 4.4*). Additionally, other life-stages used a larger proportion of PPB (KUD_{95} = 128.99 km^2), compared to neonates (KUD_{95} = 76.22 km^2) (*Fig. 4.4*). Selectivity analysis revealed a strong preference for neonates (χ^2 = 1250.53, *df* = 227, *p < 0.01*) and other life-stages ($\chi 2$ = 1907.91, *df* = 259, *p < 0.01*) to remain near receivers located in the middle of the bay (*Fig. 4.4*). Similarly when receivers were grouped into areas there was a clear preference for receivers located in the middle of the bay (Midbay) by both neonates ($\chi 2$ = 976.70, *df* = 74, *p < 0.01*) and other life-stages ($\chi 2$ = 1194.84, *df* = 51, *p < 0.01*) (*Table 4.2, Fig. 4.5*). Neonates showed the least preference for receivers in the northern area near Melbourne, South Channel and Heads receivers (w_i = 0.12, 0.18, 0.12) whereas for other life-stages least preferred receivers were located in the South Channel area (w_i = 0.38) followed by receivers in the north (w_i = 0.44) (*Table 4.2*).

Habitat preference was also reflected in depth use with both neonates (χ^2 = 913.21; *df* = 61; *p* < 0.01) and other life-stages (χ^2 = 765.85; *df* = 37; *p* < 0.01) occurring near receivers in deeper areas (>20 m) within the bay (*Table 4.3, Fig 4.6, Appendix 7.1*). Both neonates and other life-stages showed the least preference for receivers at depth 5-10m (*Table 4.3, Fig 4.6*). Other life-stages displayed high levels of movements between receivers around the bay than neonates and frequented more receivers within the bay as well as a greater variety of depths. Neonates tended to move between receivers located in the middle of bay (*Fig 4.4*). Neonates used the same depths over a 24-hour period as other life-stages (*Fig. 4.7*). Neonates showed a strong preference for depths >20 m during the day (χ^2 = 1043.69; *df* = 41; *p < 0.01*) and both 15 – 20 m and >20 m during the night (χ^2 = 716.01; *df* = 39; *p < 0.01*). Other life-stages showed a similar strong preference for depths >20 m and 15 – 20 m during the day (χ^2 = 716.00; *df* = 39; *p < 0.01*) and only for >20 m during the night (χ^2 = 1486.69; *df* = 49; *p < 0.01*)

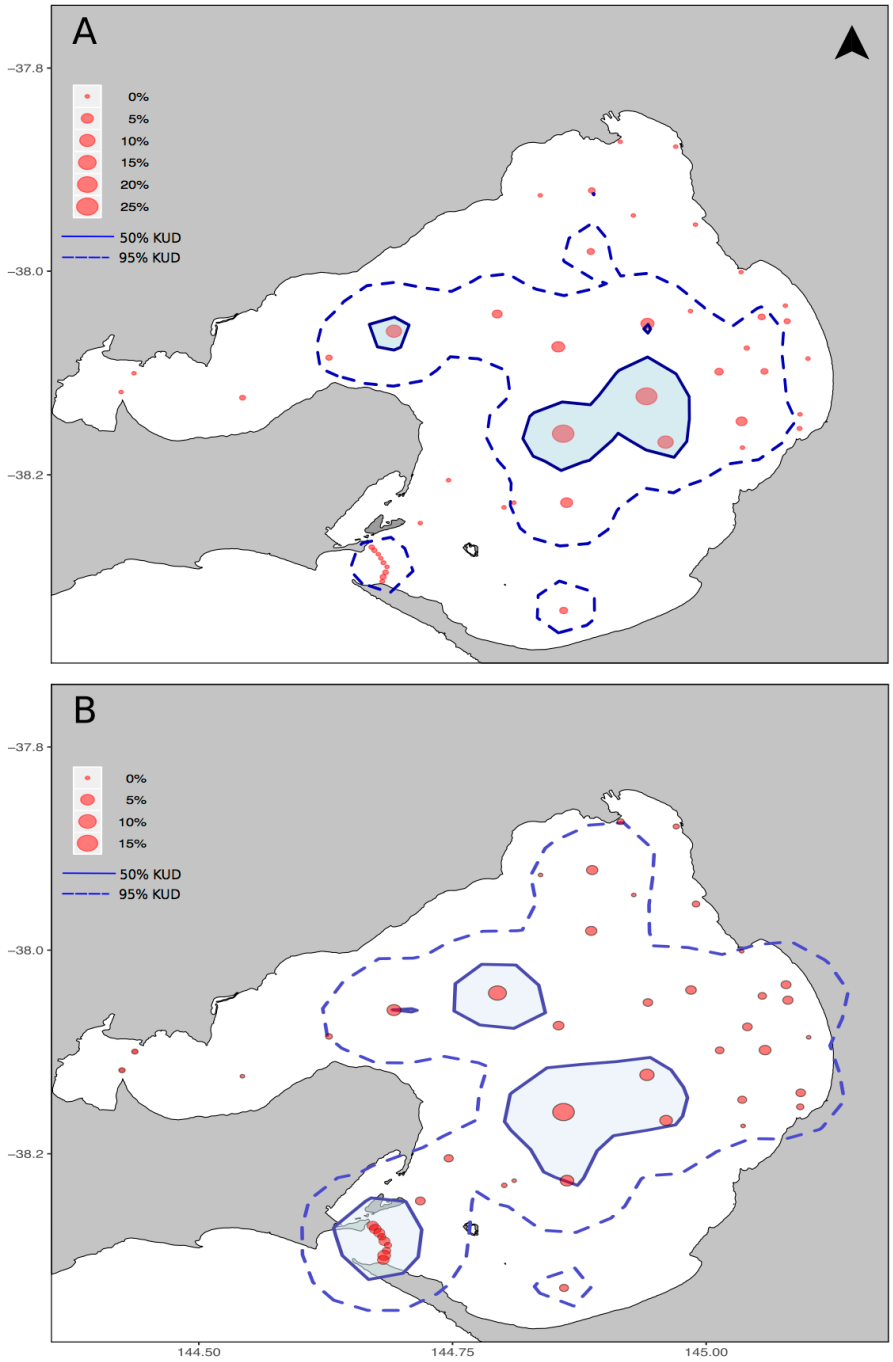

Figure 4.4 – Spatial use of Port Phillip Bay by *N. cepedianus*. A: Neonate and B: Other life-stages. Size of circle indicates the percentage of total detection per day at each receiver. Solid and dashed lines represent the overall 50 and 95% Brownian bridge kernel utilisation distribution (KUD), respectively.

Table 4.2 – Selection ratios (w_i) at areas for all sharks, neonates and other life-stages, with respective 99% confidence interval (CI) lower and upper limits.

All				
Habitat (area)	*Wi*	SE	CI lower	CI upper
East	0.48	0.11	0.15	0.82
Geelong	0.68	0.24	-0.07	1.43
Heads	0.79	0.40	-0.46	2.04
Midbay	**3.60**	0.73	1.31	5.89
North	0.34	0.13	-0.07	0.75
South Channel	0.32	0.08	0.06	0.58
Neonates				
Habitat (area)	*Wi*	SE	CI lower	CI upper
East	0.30	0.07	0.09	0.51
Geelong	1.09	0.33	0.05	2.12
Heads	0.12	0.03	0.01	0.22
Midbay	**4.83**	0.24	4.07	5.59
North	0.12	0.04	-0.02	0.26
South Channel	0.18	0.04	0.05	0.32
Other				
Habitat (area)	*Wi*	SE	CI lower	CI upper
East	0.56	0.11	0.21	0.90
Geelong	0.51	0.10	0.20	0.82
Heads	**1.07**	0.27	0.21	1.93
Midbay	**3.08**	0.48	1.57	4.59
North	0.44	0.20	-0.19	1.07
South Channel	0.38	0.09	0.09	0.68

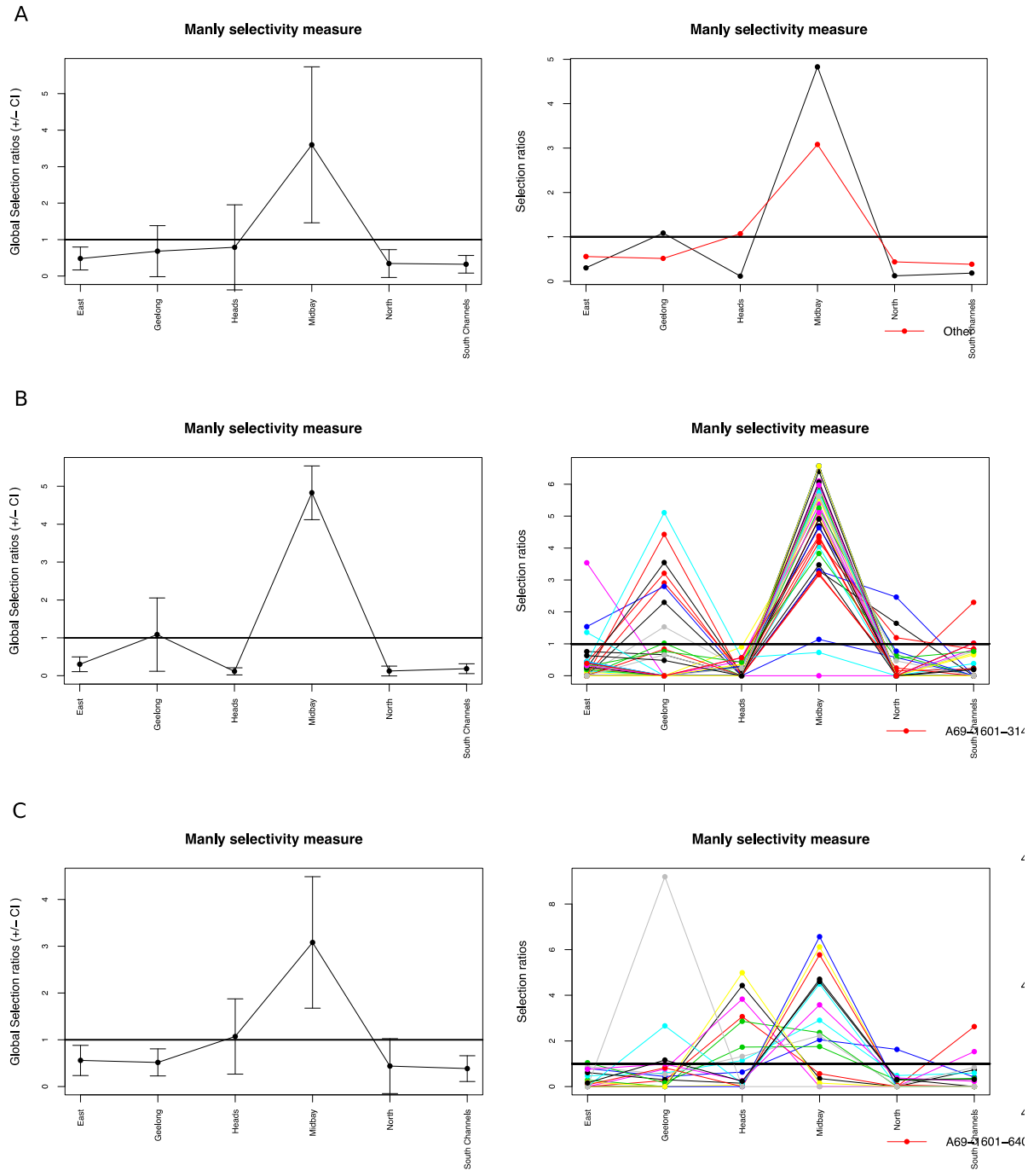

Figure 4.5 – Manly's selection ratio (Bonferroni 99% CI) for each area category. (A) Comparison of section ration between neonates (black line) and other (red line) life-stages. (B) Comparison between individual neonates. (C) Comparison between individual other life-stages.

Table 4.3 – Selection ratios (w_i) at depth for all sharks, neonates and other life-stages, with respective 99% confidence interval (CI) lower and upper limits.

All				
Habitat (depth (m))	W_i	SE	CI lower	CI upper
5-10	0.19	0.10	-0.10	0.49
10-15	0.77	0.14	0.34	1.20
15-20	0.90	0.24	0.18	1.61
>20	**3.51**	1.05	0.33	6.70
Neonates				
Habitat (depth (m))	W_i	SE	CI lower	CI upper
5-10	0.03	0.01	-0.01	0.06
10-15	0.53	0.12	0.16	0.90
15-20	0.50	0.12	0.12	0.87
>20	**5.30**	0.38	4.15	6.44
Other				
Habitat (depth (m))	W_i	SE	CI lower	CI upper
5-10	0.26	0.06	0.08	0.45
10-15	0.87	0.12	0.51	1.23
15-20	**1.06**	0.11	0.72	1.40
>20	**2.77**	0.40	1.56	3.98

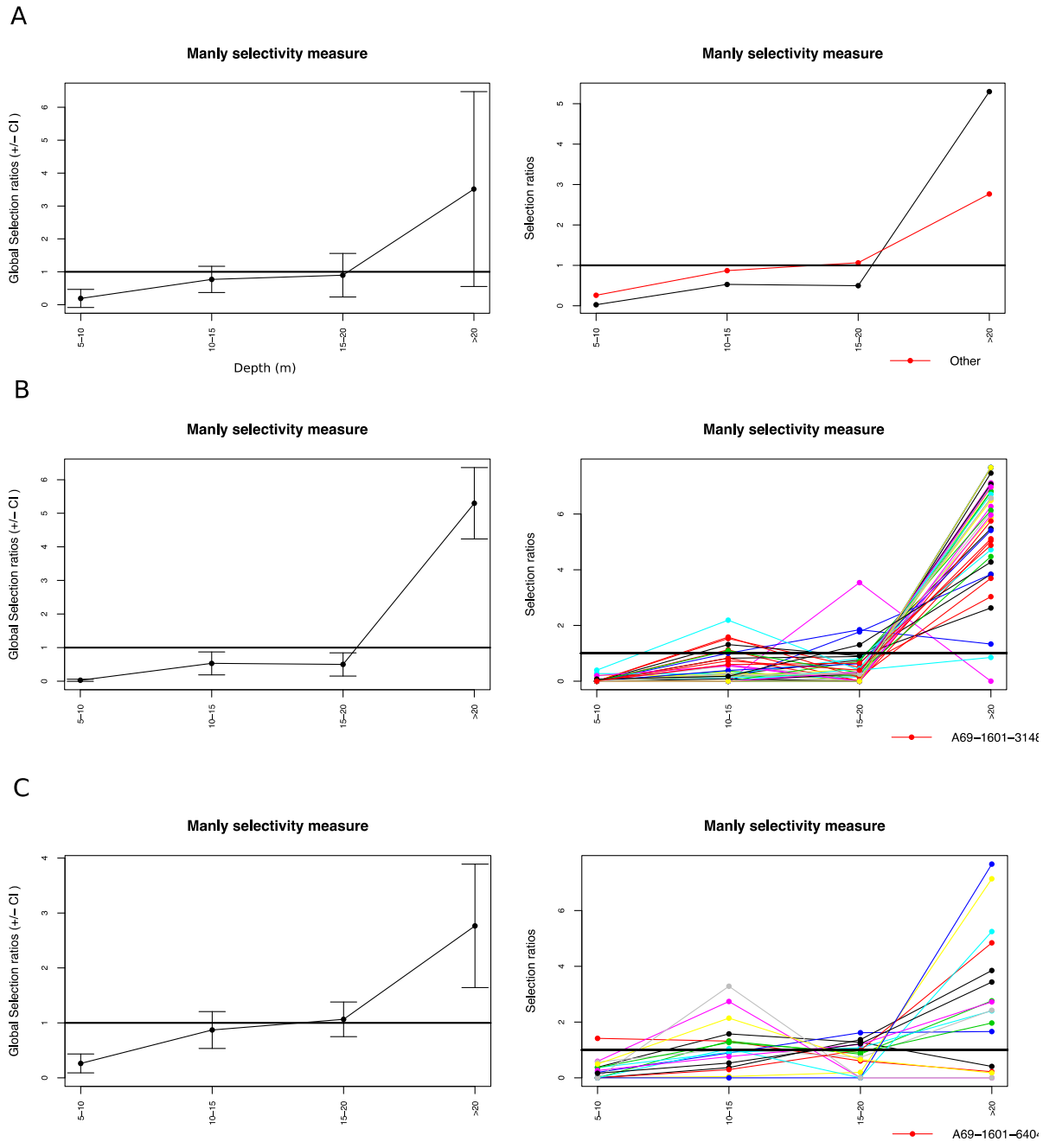

Figure 4.6 – Manly's selection ratio (Bonferroni 99% CI) for each depth category. (A) Comparison of section ration between neonates (black line) and other (red line) life-stages. (B) Comparison between individual neonates. (C) Comparison between individual other life-stages.

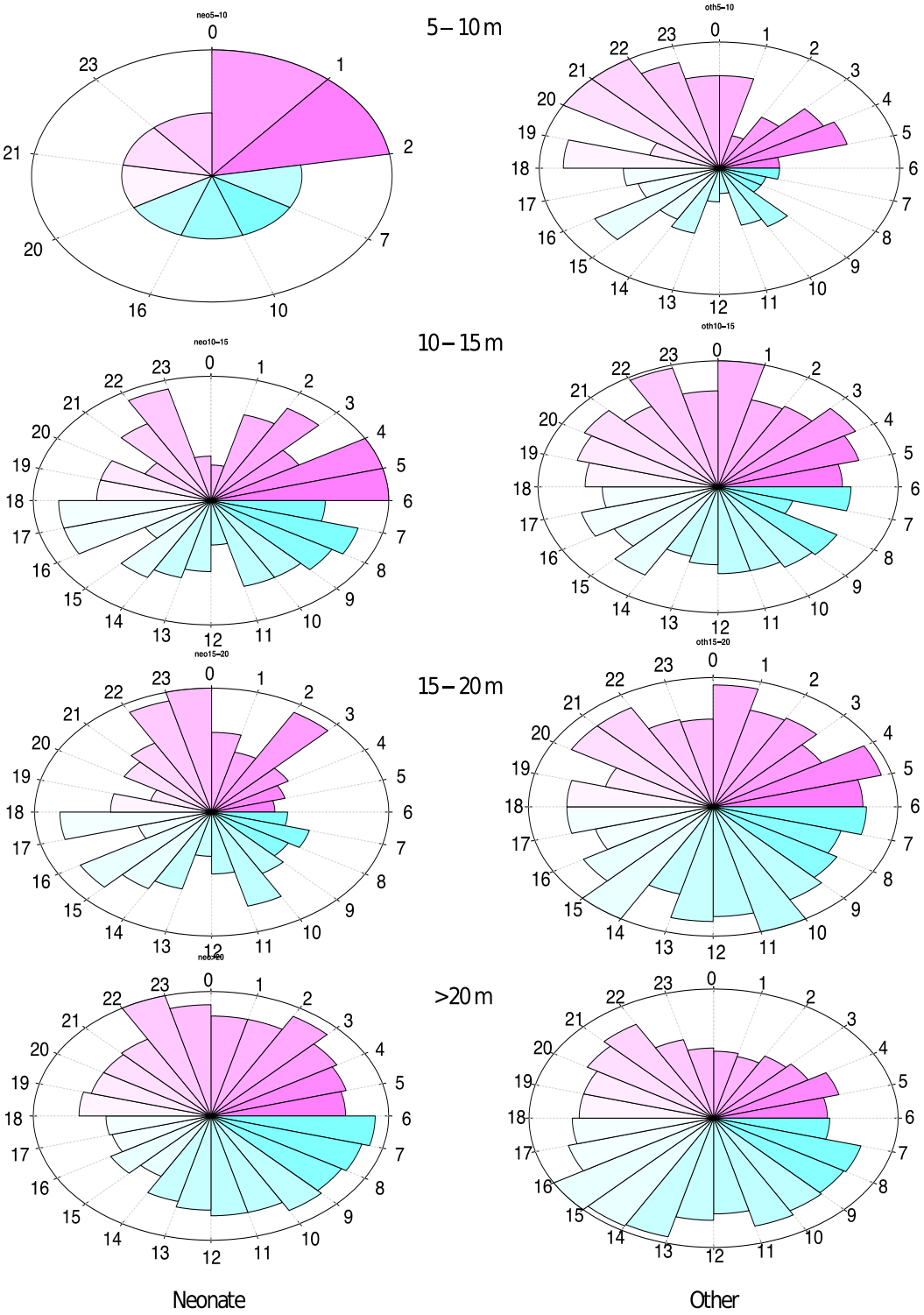

Figure 4.7 – Circular plots showing the frequency of neonate and other life-stage *N. cepedianus* detected at each hour for different depths in Port Phillip Bay. Pink represents night-time hours and blue daytime hours.

4.4.4 **Recaptures**

4.4.4.1 ***Port Phillip Bay***

Three sharks (SG15, SG10_N and SG95) were recaptured within Port Phillip Bay by fishers. These sharks were at liberty 19, 27 and 1077 days and were recaptured 23, 14, 14km respectively from the original tagging location (*Table 4.4*).

4.4.4.2 ***Long-range movement***

A total of nine sharks were detected outside PPB (eight with acoustic tags and one with a dart tag). Five sharks (SG2, SG3, SG54_N, SG38_N and S93) were detected by the IMOS receiver network (IMOS 2018) and four sharks (SG9_N, SG32_N, SG50_N, and SG58_N (only dart tagged)) were recaptured by fishers and reported (*Table 4.5*). Recaptures occurred along the coastlines of NSW, Victoria, and Tasmania (Flinders and Maria Island) (*Table 4.5, Fig. 4.8*). Recaptured sharks comprised of six neonates (three females and three males) and three other life-stage sharks (all females, 125 – 190 cm TL, one juvenile and two sub-adults). Neonates travelled distances ranging from 151 – 586 km and other life-stages from 185 – 646 km. The furthest distance travelled (646km) was to Jervis Bay in New South Wales (NSW) by a large female, 184 cm TL. The furthest distance a neonate was recorded to travel was 586 km to Maria Island in 129 days.

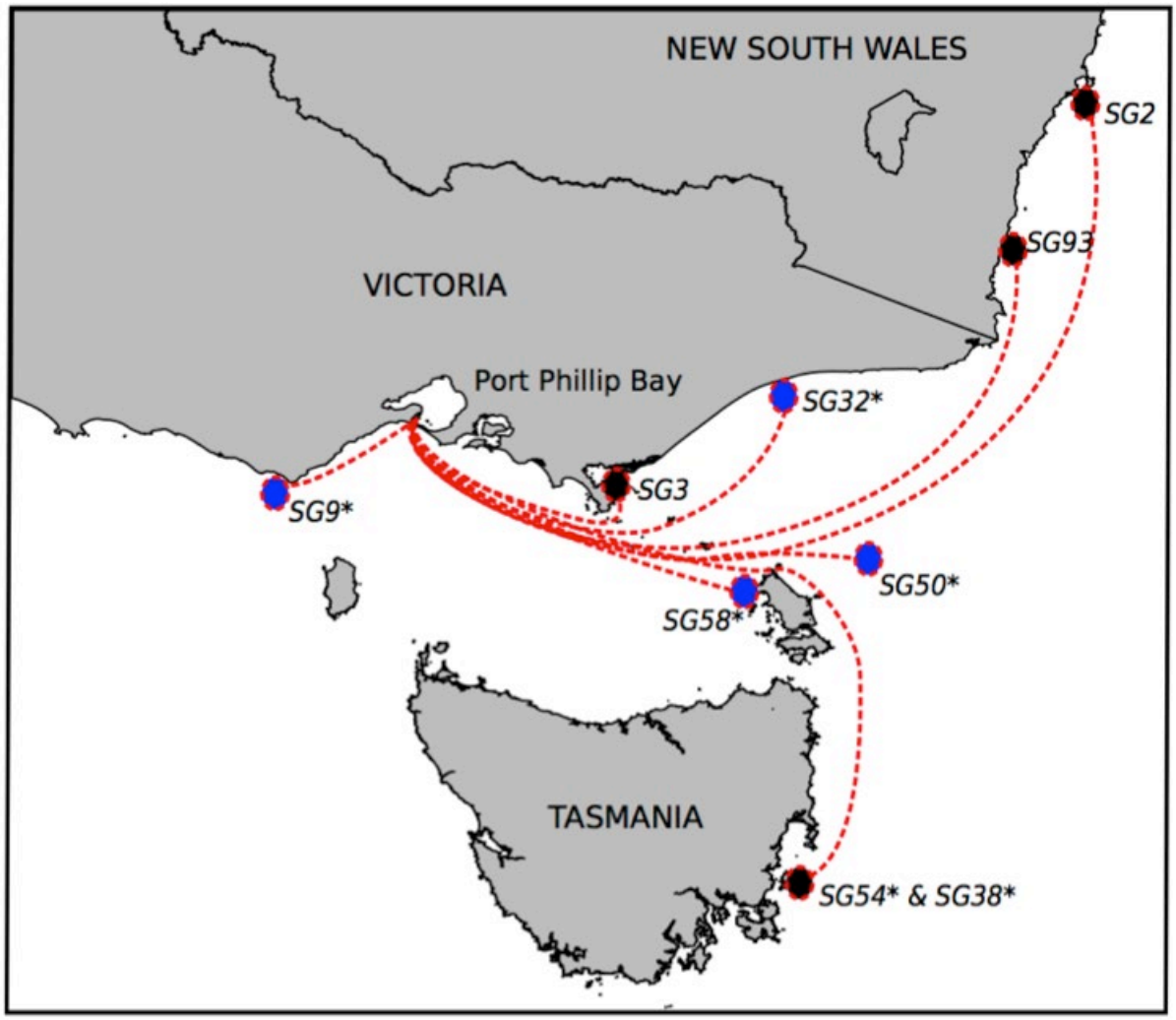

Figure 4.8 – Long-range movements from tagging location (straight travel paths) to recapture location of acoustically and fin tagged *N. cepedianus*. Detected by IMOS receiver network (black) and recaptures reported by fishers (blue). Neonates are represented by *.

Table 4.4 – Details of *N. cepedianus* recaptured inside Port Phillip Bay.

Tag	Shark	Sex	TL (cm)	Tag Date	Tag Location	Recapture Date	Days	Distance travelled (km)	Location of Recapture	Other information
14726	SG15	F	110	12/03/2015	Geelong Arm	30/03/2015	19	23	Werribee	Recaptured by fishers
31491	SG10_N	F	72	2/03/2015	MidBay	28/03/2015	27	14	Chelsea	Recaptured by fishers
64055	SG95	F	185	22/4/2015	MidBay	3/04/2018	1077	14	Symonds channel	Recaptured by fishers

Table 4.5 – Details of *N. cepedianus* recaptured outside of Port Phillip Bay.

Tag	Shark	Sex	TL (cm)	Tag Date	Tag Location	Recapture Date	Days	Distance travelled (km)	Location of Recapture	Other information
64041	SG2	F	184	26/11/2014	Port Phillip Bay	23/09/2015	301	646	Jarvis Bay (NSW)	ATTAMS
64043	SG3	F	190	28/11/2014	Port Phillip Bay	3/09/2015	279	185	Rabbit and Corner inlet (VIC)	ATTAMS
31501	SG9_N	F	60	2/03/2015	Port Phillip Bay	8/03/2015	6	151	Cape Otway (VIC)	Recaptured by fishers
31498	SG32_N	F	61.5	29/03/2015	Port Phillip Bay	3/12/2016	615	279	Lake Entrance (VIC)	Recaptured by fishers
P3161	SG58_N	M	62	10/04/2015	Port Phillip Bay	9/12/2015	243	323	West of Flinders Island (Bass Strait)	Recaptured by fishers
1463	SG50_N	M	71.5	10/04/2015	Port Phillip Bay	11/01/2016	276	360	Eastern of Flinders Island (Bass Strait)	Recaptured by fishers
1464	SG54_N	F	71	10/4/2015	Port Phillip Bay	17/08/2015	129	586	Maria Island (TAS)	ATTAMS
64057	SG93	F	125	22/4/2015	Port Phillip Bay	9/05/2016	383	516	Narooma (NSW)	ATTAMS

31500	SG38_N	M	72	30/3/2015	Port Phillip Bay	28/11/2017	974	574	Maria Island (TAS)	ATTAMS

4.5 Discussion

4.5.1 **Key habitats**

This study confirms the presence of neonate *N. cepedianus* in Port Phillip Bay. Overall the results indicate a high relative abundance of neonates in PPB during the autumn months (March – April 2015), limited residence periods and no return of neonates that departed PPB over the 2-year study period (November 2014 – 29 January 2017). Heupel et al. (2007)'s definition of a nursery area terms to identify key habitats where young sharks are present, such as pupping areas: where young are hatched or birthed. Some pupping areas may also be considered nursery areas, depending on residency and survival/growth benefits derived from their periods of residency. In accordance with Heupel et al. (2007)'s criteria for identifying a nursery area, our results suggest that PPB is not a nursery area but perhaps a pupping area for the species. Overall these terminologies do not detract from the importance of these areas toward species ecology. However, it has increasing become clear that well-protected and managed nursery areas alone cannot effectively sustain stock stability. Management and protection of breeding stock/pre-breeding stock is equally required and beneficial for the overall stock stability, conservation and management of sharks (Kinney & Simpfendorfer 2009).

Consideration must be given to selection bias towards smaller life-stages as a result of fishing gear (small hooks), although some larger sharks were also caught using this gear. CPUE result provides at minimum a preliminary insight into the relative abundance of neonate *N. cepedianus* in PPB. Telemetry data indicated that neonates tended not to reside in the bay after July 2015 and none were recorded to return to the PPB within their first 2 years. It is possible that neonates may have been present in areas of lower receiver coverage and/or tags were not detected by the entrance receivers (the Heads), due to masking (substrate, weather) or gaps in the receiver curtain. Evident by several neonates recaptured outside the bay, without being detected leaving the bay. This may also indicate that neonate sharks do not spend a lot of time near the entrance and promptly exit the bay once in the area. However, mortality may also have contributed, particularly given that natural mortality rates for juvenile sharks generally tends

to be high (Heupel & Simpfendorfer 2002, Duncan & Holland 2006) and the possibility that tagging may have contributed to some mortality (Skomal 2007), although there was no evidence for this from sharks released close to receivers.

Contrasting residency behaviour between neonate and other life-stages was also evident in this study. Other life-stages showed higher levels of residency within the bay, with 4 (24%) individuals residing in the bay up to 746 days whereas neonate sharks exhibited little to no long-term residency behaviour. Additionally, other life-stages (6, 29%) also returned to PPB after departing showing higher levels of possible philopatric behaviour than their younger counterparts. No studies thus far have specifically investigated *N. cepedianus* neonate residency and movement behaviour, however several studies have been conducted on juvenile movement in other shark species. These have often indicated higher residency behaviour, residing for months to years, and a tendency to return to these areas in subsequent years (Heupel et al. 2007, Hussey et al. 2009, Tavares et al. 2016, Oh et al. 2017). Shorter residency of juvenile sharks in an area has also been linked to changing environmental conditions, such as declining water temperature. A significant drop in water temperature in an estuarine environment resulted in the death or departures of juvenile bull sharks (*Carcharhinus leucas*) in Florida (Matich & Heithaus 2012). Port Phillip Bay water temperatures can reach a maximum 24°C in summer and minimum of 9 – 10°C in winter. During June – October 2015 water temperatures in PPB were on average 14.6 – 15.8° C (13.6 – 14.7°C minimum). Low water temperature may be a factor influencing neonate sevengill shark residency within PPB. Food abundance can be influenced by temperature changes, particularly in temperate habitats. In Tasmania, sevengill shark prey were shown to be less abundant during the winter months, coinciding with a decrease in abundance of *N. cepedianus* (Barnett et al. 2012). This may also be the case in PPB as snapper stock decrease during the winter months, which may in turn lead to younger life-stages leaving the bay during winter in search of more abundant food source.

Several studies have documented similar residency behaviour in other life-stages of *N. cepedianus*. Sevengill shark studies in Tasmania, California and South Africa have shown that

juvenile and adults sharks resided in inshore bays for extended periods (months) (Ebert 1986, 1989, Ebert 1996, Barnett et al. 2010c, Barnett et al. 2012, Barnett & Semmens 2012). Residency within these bays is influenced by sex, and environmental conditions (season, water temperature). Adult females have been shown to exhibit higher residency behaviour than males within inshore bays and *N. cepedianus* tended to be more abundant within inshore bays during the spring-summer months, exhibiting seasonal site-fidelity (Ebert 1989, Ebert 1996, Barnett et al. 2010c, Williams et al. 2011, Barnett et al. 2012, Williams et al. 2012, Ketchum et al. 2017). This type of seasonal movement has been linked to feeding behaviour (prey movement and abundance). For example in southern Patagonia (Argentina) and south-eastern Tasmania (Australia), the abundance of *N. cepedianus* (>100 cm TL) in coastal bays peak in summer, corresponding with an increase in prey (Cedrola et al. 2009, Barnett et al. 2011, Barnett & Semmens 2012). A lack of neonates or young-of-the-year (<100 cm TL) observed in these areas, suggest they are unlikely pupping or nursery areas. Some studies have suggested the use of coastal bays as pupping and nursery areas for this species but these were bases solely on the presence of some young sharks (Ebert 1989, Lucifora et al. 2005).

Coastal bays and estuaries are thought to be advantageous for survivorship of young shark by providing an abundance of food, and protection from predation (Heithaus 2007, Heupel et al. 2007). Port Phillip Bays is a communal nursery area for a variety of species including for school sharks (Stevens & West 1997), as well as teleosts such as snapper (*Chrysophrys auratus*) (Hamer & Conron 2016, Hamer & Mills 2017). The bay also retains an abundance of food sources, such as a variety of invertebrates, teleost, elasmobranchs (including small sharks), and marine mammals, all of which comprise the diet of adult *N. cepedianus* (Ebert 1991, Lucifora et al. 2005, Braccini 2008, Barnett et al. 2010a). Adult snapper are most abundant during the late spring to early summer months (October to December), which may explain the detection of adults in the bay during these periods. Dietary analysis of juvenile (<120 cm TL) *N. cepedianus*, off southern Africa and California, USA, revealed the consumption of primarily teleost's (Ebert 2002). Smaller juvenile and sub-adult (Pinky) snapper (15 – 35 cm TL) are most abundant during the late summer – autumn (February – May), which corresponded with the months of highest relative abundance of neonates within PPB. This may provide an

abundance of food for younger life-stages, thereby increase survivorship and growth. Additionally, dietary partitioning of adults and sub-adults has been observed in *N. cepedianus* and has been suggested to reduce conspecific competition and/or predation, possibly increasing the survivorship of younger life-stages (Ebert 2002, Cedrola et al. 2009). This may also apply to *N. cepedianus* in PPB resulting in reduced predation and competition with neonates due to their dietary difference and increase survivorship of this early life-stage in PPB.

4.5.2 **Movement and spatial use of PPB**

An overlap in habitat and depth use of *N. cepedianus* neonates and other life stages was observed, with a particular preference for deeper areas (>20 m) in the middle of PPB. Although there was a significant overlap in habitat and depth use of the life-stages, larger sharks tended to utilise greater spatial areas within the bay and were detected at more receivers than smaller sharks.

Distribution of sharks in an area can often vary spatially according to age, size and sex. Intra-specific predation or cannibalism of juveniles by larger conspecifics can influence juvenile distribution and habitat use in area (Guttridge et al. 2012). This type of behaviour has been observed in several species of shark including bull sharks (*Carcharhinus leucas*), lemon sharks (*Negaprion brevirostris*) and great hammerhead sharks (*Sphyrna mokarran*) (Morrissey & Gruber 1993, Duncan & Holland 2006, Heupel & Simpfendorfer 2011, Guttridge et al. 2012). For examples, sub-adult lemon sharks (>1.3m TL) have been observed preying on juvenile lemon sharks (<1 m TL) (Morrissey & Gruber 1993). Juvenile sharks have also been shown to use shallower areas as refuge from intra-specific predation (Heupel & Simpfendorfer 2011, Guttridge et al. 2012). The opposite was observed for neonate *N. cepedianus*, as they seemed to avoid shallower areas and preferred deeper areas (>20 m) of the bay, similar to larger conspecifics. Why precisely juvenile movement was limited to deeper areas of the bay is unknown but possible theories could be the availability of food, and predation avoidance. It

has been speculated that shallow areas may not provide the most effective evasion tactic as it limits a preys escape options, whereas in deeper areas there is an additional escape dimension of vertical (up/down) movement (Andrews et al. 2009, Andrews et al. 2010). Additionally, visibility tends to be negatively correlated to depth, with decreases in visibility with increasing depth. Thus murky, low visibility water conditions may assist in reducing predation risk. Diel behaviour of neonates and other life-stages showed overlapping depth preference in PPB. Neonates exhibited strong preferences for deeper areas > 20 m during the day while their preference expanded to shallower areas, 15 – 20 m during the night in PPB. Contrastingly, other life-stages exhibited larger diel depth preference during the day (15 – 20 m and > 20m) while preferences narrowed during the night to > 20 m depth. Diel foraging behaviour has been shown for *N. cepedianus,* with active nocturnal foraging in deeper areas and opportunistic foraging during the day in shallower areas (Barnett et al. 2010b). The movement of neonate sharks to shallower areas at night may be in response to increased predatory behaviour by larger conspecifics in deeper areas. This may reduce the conspecific predation risk on smaller *N. cepedianus*. During fishing activities, several neonate *N. cepedianus* were caught in the same geographical location (*Fig 4.2*), which may indicate aggregation behaviour. Several species of sharks have also been shown to display aggregation behaviour to reduce predation risk (Heupel et al. 2007).

Juvenile sharks have also been shown to use less spatial area than their adult counterparts. Movement of adult and juvenile white sharks (*Carcharodon carcharias*) showed a positive correlation between total length and space use with smaller sharks utilising a smaller core area than larger sharks (Hoyos-Padilla et al. 2016). Sevengill shark neonate and other life-stages exhibited similar patterns within PPB with area use increasing with size. Blacktip shark juveniles were shown to increase their area of use over time, thus an increase in size positively correlated with an expansion in area use (Heupel et al. 2004).

4.5.3 **Long-range movement**

Recapture information indicated connectivity between State jurisdictional waters, with both neonate and older life-stages moving between Victoria, Tasmania, and New South Wales. The furthest distances travelled by neonate and older life-stages were 586 km and 649 km to TAS and NSW respectively. Similar long distance movement has been recorded for this species between coastal bays along the western coast of North America, from Washington to California (Williams et al. 2012, Ketchum et al. 2017). Similarly, *N. cepedianus* adults tagged in south-eastern Tasmania travelled to the Bass Strait and New South Wales (Barnett et al. 2011, Stehfest et al. 2014). Though there were no recaptures of tagged *N. cepedianus* in South Australia this does not exclude that juvenile sharks may be travelling there. Indeed, one shark tagged in this study was recaptured having moved several hundred km to the west of PPB. Movement over large spatial scales provides an opportunity for the broad-scale exchange of genetic material. The genetic analysis results of *Chapter 3* showed little genetic structuring among the sevengill shark populations of south-eastern Australia, indicative of high genetic connectivity and mixing between the populations. Long distance and local movement help link different ecosystem and facilitate the transfer of energy among them (Jeltsch et al. 2013, Dulvy et al. 2017). Thus, the use of genetic and telemetry data have indicated that *N. cepedianus* in south-eastern Australia comprise of one genetically and reproductively mixing population and thus should be managed as such across state boundaries.

4.6 Conclusions

This study identified the first pupping area for *N. cepedianus* in Australia. Port Phillip Bay appears to be an important habitat for all life stages and reproductive behaviour (pupping). This is a significant finding for the understanding of habitat use by *N. cepedianus* from different life-stages. Understanding habitat use at different stages of the life cycle is important for management, particularly species impacted by fishing. Further research is required to determine if there are other pupping and/or nursery areas for *N. cepedianus* in Australia.

Neighbouring bays to PPB, such as Western Port Bay and Corner Inlet may also be important habitats for *N. cepedianus*. Additionally, anecdotal reports of fishers from 2006 indicated high catches of *N. cepedianus,* 70 to 90 cm (TL), in the Bass Strait, northern (pers. Com. *Dr. Adam Barnett with commercial fisherman*). This may be an important habitat for juvenile *N. cepedianus* and represent a nursery or second pupping area for south-eastern Australia.

Additionally, considering that female *N. cepedianus* in south-eastern Tasmania exhibited high site fidelity to coastal bays, while males where shown to exhibit long distant migration within the region. This may indicate that gene flow and connectivity within south-eastern Australia may be largely facilitated by male movement. Further genetic analysis on the kinship of neonates from PPB to the wider stock would provide additional insight on stock replenishment. Findings from *Chapter 3* indicate that *N. cepedianus* in Australia comprise one interconnected and mixing stock. Thus, determining if PPB is the main pupping ground and identifying other possible key reproductive areas for *N. cepedianus* in south-eastern Australia is very important for establishing a protocol for the management of this species in the region.

5 General Discussion

A population is defined as a group of interbreeding individuals within a geographic area, that may also incorporate subpopulations (Frisk et al. 2014). The identification of subpopulations and an understanding of the connectivity within a metapopulation (a group of spatially separated conspecific populations) is the basis for any management and/or conservations plans. Answers to questions such as; What population/subpopulations need to be managed? and; Where are they located and what are their boundaries of distribution?, are required. However, the ecological information necessary to answers these questions are often unavailable for many shark species. The term population structure encompasses a variety of ecological information such as, sex and age ratios, as well as genetic structure, which looks at variation within and between populations. This information can be used for stock assessments and provides insight into the population health and stability of a species (Palsbøll et al. 2006, Reiss et al. 2009, Spaet et al. 2015).

Global trends over the last several decades show a decline in shark stocks, primarily in response to increased exploitation (fisheries), habitat degradation/loss and pollution (Dulvy et al. 2014). Considering that approximately 49% of shark species are listed as "Data Deficient" by the IUCN, there is a pressing need for basic ecological and stock structure data to aid in the development of sustainable management and conservation strategies (Musick 2005, Musick & Bonfil 2005). Shark research has tended to be biased towards commercially important, often coastally associated species, due to their accessibility and abundance in fisheries (Walker 1998, Dulvy et al. 2014). Advancements in technologies for tracking movement (acoustic, satellite, and video telemetry) (Heupel et al. 2006, Block et al. 2011) and genetic techniques (microsatellites, genotype by sequencing methods) (Palsbøll et al. 2006, Dudgeon et al. 2012,

Larson et al. 2017) have greatly increased our ecological understanding of more elusive shark species, in less accessibly habitats (pelagic, temperate and deep sea). For example acoustic tagging has shown philopatry, seasonal migratory and diel movement pattern for a variety of shark species (Ketchum et al. 2014, Acuña-Marrero et al. 2017, Skomal et al. 2017). Genetic analysis has improved our understanding of populations structure and connectivity by identifying subpopulation, speciation and diversity within and between populations (Bernard et al. 2016, Camargo et al. 2016, Maduna et al. 2017, Veríssimo et al. 2017). Combining methodologies provides a wealth of information that can be used to assess stock structure, identify key habitats and threats to population stability.

The broadnose sevengill shark, *Notorynchus cepedianus* is an important temperate coastal associated apex predator. However, this species is one of the many listed as data deficient by the IUCN. The low commercial value of *N. cepedianus* has resulted in limited fisheries data for this species across its global distribution with some reported regional management (Cedrola et al. 2009, Barnett et al. 2012, De Wysiecki et al. 2018). However, *N. cepedianus* low fecundity and life history traits suggest it may be susceptible to fishing pressure. Major gaps in knowledge currently persist with respect to global and local population structure and connectivity, identification of key habitats and the early life-stage behaviour. The absence of this information has hindered stock assessments and threats to this species worldwide remain unclear. Considering this species coastal distribution, anthropogenic factors such as fishing pressure, habitat degradation/loss and pollution may have negative impacts on their population (Suchanek 1994). This thesis aimed to provide information on the population structure and connectivity (globally and regionally) and identify a key reproductive habitat through the monitoring of juvenile movement. The results of this study could be used to assist in the development of stock assessment and the establishment of management plans.

This study also emphasises the importance of a multifaceted approach to understanding basic ecology of organisms. This project utilised a combination of conventional tag-and-release and acoustic tracking to assess movement patterns and identify key habitats, with state-of-the-art molecular technologies to infer levels of population structure, connectivity and standing

genetic diversity on a global and regional scale (south-eastern Australia) for *N. cepedianus*. The interdisciplinary analyses complemented each other and draw a more comprehensive picture of interregional structure (oceanic basin delineations), intraregional connectivity and the identification of a key habitat (pupping area) for *N. cepedianus* in south-eastern Australia.

5.1 Global genetic structure

Mitochondrial markers are informative for providing information on population divergences along evolutionary time scales however, fall short to resolve divergence along ecological time scales. Furthermore, mitochondrial markers tend to reflect maternal divergences but fail to reflect paternal divergences (Hueter et al. 2005, Dudgeon et al. 2012). In this study, mitochondrial markers showed clear separations across oceanic regions and the identification of three distinct groups: Eastern Pacific Ocean (EPO), South Atlantic (SAO) and Oceania were obtained. However, limited genetic differences or diversity were detected within populations. Depending on the definition and criteria for speciation, we conservatively suggest that three distinct clades (EPO, SAO and Oceania) are present. This information was previously unknown and *N. cepedianus* was considered to be one genetically interconnected population. However, this preliminary analysis indicates that the differentiation between the regions is not significant enough to warrant classification as a separate species at this time. Notwithstanding, further study is required to fully understand these regional *N. cepedianus* clades. Considering the fluidity of classification concepts at present, the global taxonomy of *N. cepedianus* may warrant re-evaluation in the future.

With the increasing number of genomic studies and the advancements in genetic technology, a diversity of population structuring of widely distributed shark species has been unearthed. Trends of oceanic separation of populations has been shown for other widely distributed sharks species such as scalloped hammerhead shark, tiger shark, shortfin mako, pelagic thresher shark and white shark, with particular geographical divisions between oceanic basins such as; between the Atlantic, Pacific, Indian and Oceania; as well as within ocean basins, for examples

between eastern and western Pacific (Heist et al. 1996, Duncan et al. 2006, Cardeñosa et al. 2014, O'Leary et al. 2015, Bernard et al. 2016, Holmes et al. 2017). These divisions have been linked to historical geological processes, such as; glaciation, continental shift, water temperature and landmass barriers, which may have created physical barriers separating the geographical populations over time, leading to genetic isolation (Cowman & Bellwood 2013, Chabot et al. 2015, Domingues et al. 2017). This study suggests that biogeographic barriers such as; closure of the Isthmus of Panama, Eastern Pacific Barrier, sea surface temperature and site fidelity may have influenced historical and current population structure of *N. cepedianus* across its global distribution.

This type of research has once again shown that widely distributed sharks species thought to be one large population are actually comprised of several smaller populations with little to no connectivity, which need to be managed as separate management units. This has reiterated the fact that further phylogenetic research is needed to uncover the hidden population structure of widely distributed species to provide accurate information on population size, structure and connectivity. This is the only way that effective management plans can be developed and implemented.

5.2 Australian genetic structure

An understanding of interregional/local population structure is also important for understanding the distribution and connectivity of populations within an area. In contrast to global distribution trends, structuring of populations within a region is often more affected by species behavioural factors, such as philopatry and site-fidelity, than biogeographic processes. Affiliations to particular habitats, such as pupping and nursery areas, can subdivide populations and limit possibilities for mixing. Evidence of philopatry in shark species has been mounting and suggested to affect population structure resulting in delineations across nursery areas, often with some mixing (Chapman et al. 2015).

The advantages of Genotype by sequencing (GBS) techniques, such as the here applied RAD Sequencing are that they can; provide a vast quantity of genomic information (adaptive divergence and identification of genomic regions), can be used for small sample sizes and demographic parameters can be estimated with greater accuracy (Larson et al. 2014). However, genomic data on its own fails to resolve contemporary patterns of species distribution and connectivity, only in combination with movement data can current stock structure be revealed (Jeltsch et al. 2013). Within Australia our results indicated minimal levels of population structuring of *N. cepedianus* across the five locations (VIC, NSW, VIC, STAS and NTAS). This was indicative of strong connectivity between the areas and evidence of mixing between the populations. Combining these results with previous movement studies, including the acoustic tracking data from *Chapter 4*, a better representation of *N. cepedianus* stock structure was revealed in Australia. Several intra-regional (within oceanic regions) genetic studies have been conducted, mainly on tropical coastal species, with varying levels of structuring depending on species and philopatric behaviour. Genetic analysis of tiger shark (*Galeocerdo cuvier*) and scalloped hammerhead shark (*Sphyrna lewini*) populations within the Indo-Pacific revealed low intra-regional structuring and high connectivity between locations, indicating that stocks should be managed across jurisdictional boundaries (Daly-Engel et al. 2012, Holmes et al. 2017). Similarly, genetic homogeneity within and between nursery areas in the Gulf of Mexico and Atlantic coast of Florida were observed for bull shark populations in the US (Sandoval Laurrabaquio-A et al. 2019). The results of *Chapter 4* also highlighted the importance of juvenile based sampling to reveal site fidelity behaviour and suggested that gene flow between regions was primarily mediated by adult males. Female driven parturition philopatry has been observed in blacktip sharks, resulting in the detection of genetic population differences between nursery areas in different regions (Keeney et al. 2005). Genomic analysis of *N. cepedianus* populations off the eastern coast of the USA, revealed genetically distinct subpopulations between coastal bays in California and Washington (Larson et al. 2015). *N. cepedianus* have been shown to exhibit seasonal site-fidelity towards coastal bays for feeding purposes (Barnett et al. 2012). Females have also been suggested to exhibit a higher degree of site-fidelity toward these bays than males (Barnett et al. 2011). The SNP markers used in the Australian genetic study (*Chapter 3*) provides a broader genomic range and the sampling of

juveniles may have offset any maternal genetic bias. It is unclear as to why there is contrasting intra-regional genetic structuring observed between *N. cepedianus* populations in the US and Australia. Male *N. cepedianus* have been shown to be more migratory in Australia, traveling between Tasmania, Victoria, and New South Wales (Stehfest et al. 2014). Thus, *N. cepedianus* population structure and connectivity in Australia may be driven by male-mediated distribution, similar to patterns observed for bull and scalloped hammerhead sharks.

Overall this chapter has illustrated the connectivity within the Australian population of *N. cepedianus* and that the main possible driver behind this connectivity is male facilitated gene flow. Additionally, movement studies of *N. cepedianus* have shown they travel distances up to ~1800km between coastal bays (Barnett et al. 2011, Williams et al. 2012, Ketchum et al. 2017), which provides physical evidence of population connectivity, corroborating the genetic results presented above.

5.3 Key habitats and movement

Acoustic tagging can resolve temporal movement patterns or residencies, however, the price of tags in addition to receiver maintenance efforts and costs limit the deployment at larger scales. This study used acoustic telemetry movement data to determine the reproductive importance of Port Phillip Bay (PPB) for *N. cepedianus.* The abundance of neonates during the autumn months, lack of residency and site fidelity behaviour, ruled out PPB as a nursery area but fit the criteria for a pupping area. This study identified the first pupping area for this species in PPB, Australia. Preliminary results indicate that this area is used by individuals from different locations within south-eastern Australia. This emphasises the connectivity within the Australian population and the possible reproductive importance of this bay for population stability. The identification of key habitats such as breeding and feeding grounds is import for understanding population structure as they can provide information on movement patterns. Nursery/pupping areas are important for maintaining populations structure and they may increase the survival rate of young individuals during their early years (Heupel et al. 2007).

However, management strategies must include plans for the protection of all life-stage classes. Considering that all life-stages spend the majority of their time outside the nursery/pupping areas, management and protection strategies should not be limited to only coastal bays. Additionally, depletions of adult stocks have been shown to have negative effects on breeding populations and recruitment size (Heupel et al. 2007). Studies have shown that the protection of only one life-stage is ineffective in the overall sustainability of a population and reiterates the need for more comprehensive thinking and management strategies (Kinney & Simpfendorfer 2009).

Analysis of movement patterns of neonate (< 80 cm) *N. cepedianus* within the PPB showed a preference for deeper areas of the bay which was surprising as this overlapped with other life-stages (> 80 cm, including juveniles, sub-adults and adults). It has been a long standing theory that nursery/pupping areas provide protection during these early developmental stages and that shallower more complex habitats such as mangroves provide the best protection from predation (inter – and intraspecific) (Guttridge et al. 2012). This however was not the case for neonate *N. cepedianus,* which occupied the same areas as their adult counterparts. It is unclear as to the exact reasons for this phenomenon but we suggested it may be as a result of the following conditions; the depth of the bay provided a third dimension (depth) for evading predation, temperatures within the bay were most stable within the deeper parts of the bay, and/or there was an abundance of food within PPB, reducing the risk of conspecific predation. It is clear that further research is needed to verify these speculations; however this study provides a preliminary look into the behaviour of neonate *N. cepedianus*, which has been unavailable in previous studies.

Tag and release tagging campaigns, are effective for receiving movement information at larger scales. They however rely on large samples sizes, the cooperation of the local fishing industry partners, and are very labour-intensive, which can limit the number of tags deployed in a study. The combination of tag and release and acoustic tagging increase the likelihood of sharks being recaptured and allows shark movement to be monitored while at liberty. The use of the data sharing platform, such as the Animal tracking Facility, Integrated Marine Observing System

(IMOS) also enabled this study to expand its detection range to most of south-eastern Australia, including the Bass Strait, north-eastern Tasmania and the NSW coastline. Long-distance movement results from this study showed both neonate and other life-stages tagged in PPB traveling to other state waters such as NSW, TAS and VIC. This further reiterates the connectivity of this Australian population and provides additional evidence toward the results of *Chapter 3* of this thesis, which revealed strong genetic connectivity between *N. cepedianus* sampled from Port Phillip Bay, Victoria, northern and south-eastern Tasmania and South Australia.

5.4 Ecological and management importance

N. cepedianus are ecologically important apex-predators in coastal ecosystems with similar trophic values as tiger and white sharks (Barnett et al. 2012, De Wysiecki et al. 2018). The removal of top predators in ecosystems have been shown to have trophic cascading effects (Myers et al. 2007, Silliman & Angelini 2012, Kotta et al. 2018). These effects disrupt the food web by decreasing the predation rate on prey, resulting in their increased population/overpopulation. This phenomenon intern has negative effects (population decrease) on the primary consumer (>3 trophic levels) or primary producers (3 trophic levels). A common example for the terrestrial environment was the removal of wolves (*Canis lupus*) from Yellowstone Park. In this tri-trophic system, this resulted in the overpopulation of elk (*Cervus elaphus*), which has a detrimental impact on the plant species within the park (Ripple et al. 2001, Ripple & Beschta 2012, Ritchie et al. 2012). Similar effects have been shown in the marine environment, with the most well known example in the Pacific kelp forests, where the removal of sea otters resulted in sea urchin overpopulations and the loss of kelp species (Estes & Duggins 1995). Reductions in shark populations has also been shown to result in food-wed disruption, ecosystem instability and mesopredator overpopulation (middle tropic level predator), which intern increase predation on lower tropic organisms (Myers et al. 2007, Prugh et al. 2009). *N. cepedianus* consume a variety of prey, including small sharks (genus *Mustelus*), batoids, teleosts and marine mammals (pinnipeds) (Ebert 1989, Ebert 1991, Lucifora et al.

2005, Braccini 2008, Barnett et al. 2010a). Many of the species consumed by *N. cepedianus* are commercially important species such as gummy shark (*Mustelus antarcticus*), school sharks (*Galeorhinus galeus*), and snapper (*Chrysophrys auratus*). Thus, a decline or loss of *N. cepedianus* from coastal areas could have top-down effects on both the ecosystem and fisheries.

Similar to other large sharks, *N. cepedianus* exhibit low fecundity rates, with slow growth, maturity and reproductive rates, which render them vulnerable to exploitations (Musick 1999, Smith et al. 1999). Globally, there has been a growing concern over the by-catch of elasmobranchs in fisheries (Cosandey-Godin & Morgan 2011, Molina & Cooke 2012, Worm et al. 2013). Due to *N. cepedianus* coastal association in temperate waters, this species is exposed to intensive inshore fisheries such as gillnet, long lining, trawling and recreational, over most of its range, including Argentina and Australia, and could be susceptible to local depletion (Zhou et al. 2007, Cedrola et al. 2009, Zhou et al. 2009, De Wysiecki et al. 2018). Nonetheless, consistent and accurate fishery landing data for this species is limited and regional stock assessments are needed across the *N. cepedianus* global range. Decreasing abundances of *N. cepedianus* have been reported in Argentina, where by-catch (trawl and gillnet) and recreational fisheries have been identified as threats to the species (Barbini et al. 2015, Irigoyen & Trobbiani 2016). In Australia this species is not targeted and is considered to be of low commercial value, thus fisheries may not be an immediate threat to this species in this region. However accurate catch data (commercial and recreational) for this species is required to effectively determine this species abundance within the fisheries.

The results of this thesis suggest that management of *N. cepedianus* should focus on regional populations, as this species seems to be separated on an inter-regional geographical scale and connected on an intra-regional scale. This thesis highlights the need for a data collection protocol for *N. cepedianus* in Australia and across its global distribution. In Australia, an assessment of fisheries-related mortality rates is required to ascertain the stock structure and effects of fisheries on this species. Additionally, we suggested that the protection of key coastal areas (pupping and feeding) and pre/breeding stocks could be an effective method for sustainably managing this species in Australia. Protecting feeding/pupping areas would

encompass both early life-stages and breeding females, while protecting pre/breeding stocks would also include large males, which have been shown to maintain gene flow and connectivity within the region. This would assist in determining the conservation status of *N. cepedianus* and ensure the sustainable management of this possibly vulnerable apex predator in Australia.

5.5 Future studies

This thesis is the first study to:

- Investigate the global phylogenetic structure of *N. cepedianus*
- Investigate the genetic structure and connectivity of *N. cepedianus* in Australia
- Identify a key juvenile habitat and understand neonate movement of *N. cepedianus* in Australia

Thus, the information presented in this thesis constitutes an essential component of future work and will be helpful for 1) understanding the global population structure, connectivity and dynamics of *N. cepedianus,* 2) as an example of the importance of multi-disciplinary approaches for understanding complex ecological process and 3) understanding the importance of early life-stage behaviour and movement to population structure and proliferation. However, there is still a need for future research to fill the gaps in knowledge and assist in a better understanding of overall ecological processes. Below outlines a few areas that require further study.

Further genetic and taxonomical review *Notorynchus cepedianus* is suggested to determine the extent of distinction between populations from the Eastern Pacific, Southern Atlantic and Oceania. Considering this species has not been documented to exhibit trans-oceanic movement, this may be grounds for isolation by distance speciation effects. Additionally genetic techniques are increasingly becoming less expensive and provide greater variety and quantity of information. Genetic sampling is also a quick and non-lethal way of obtaining large

quantities of biological information. Further mitogenome comparisons of newborn /juveniles and adults from different sites (within regions) could provide insight into the extent of site-fidelity, possible natal philopatry and genetic connectivity between areas. Thus additional genetic sampling within and between regions is advised. Long-term and long distance movement studies within regions could provide much needed information on the spatial boundaries of this species across the globe. Considering that there was no genetic distinction between populations from north-eastern (USA) and south-eastern Pacific (Peru) region, movement studies to determine if these intra-regional areas are physically connected would significantly contribute to our understand of this species ecology and spatial boundaries. The use of satellite tags and collaborative acoustic networks (similar to IMOS in Australia) would be beneficial for long distance studies and to help understand migratory patterns in the face of climate change (Robinson et al. 2009, Abecasis et al. 2018). Further investigation into neonates and juveniles behaviour and movement is warranted and would help to identify other key habitats, such as nursery/pupping areas in Australia and other regions. Anecdotal reports of neonates suggest that such studies should be conducted in South Australia and Northern Tasmania (Bass Strait). Additionally, the nursery and pupping area definition proposed by Heupel et al. (2007) should be adopted for further studies to provide a standardised method and possibility for comparisons.

A fisheries assessment of catch and mortality rates for *N. cepedianus* is urgently needed to determine its conservation status and identify possible threats to this species population stability on a regional level. Though this species is considered to be of low economic value, they are still present in finfish and shark fisheries as a bycatch. It is unclear the as to the extent of catch and mortality this may represent for local and global population of *N. cepedianus*. Thus catch and mortality records are required to determine the effect fisheries my have on this species population stability. Additionally, genetic sampling methods should be adopted into these fisheries assessment protocols.

Port Phillip Bay is an ecologically productive area, important to several fisheries. Determining the role of *N. cepedianus* as an apex-predator and how that affects interactions/relationships with mesopredators would assist in determining their influence on the ecosystem structure and

dynamics of Port Phillip Bay. Additionally, identifying physical and ecological benefits this bay may provides for early life stages of *N. cepedianus* could help identify similar areas as pupping or nursery regions.

This study provides novel information on the global and Australian genetic population structure and identifies a pupping area for the broadnose sevengill shark (*N. cepedianus*), an important temperate coastal apex-predator.

6 Appendix

Appendix 7.1. Receiver information

Receiver Name	Latitude	Longitude	Depth (m)	Depth Category (m)	Region
01 North Gellibrand	-37.8770167	144.9147167	10	<5-10	North
03 Point Ormond	-37.881717	144.969233	5	<5-10	North
04 Dumb Joe	-37.929017	144.836117	11	10-15	North
05 North P2	-37.924267	144.886733	17.9	15-20	North
06 Faulkner Beacon	-37.948583	144.927583	15.5	15-20	North
07 North Spoilground	-37.983583	144.8858	18.6	15-20	North
08 Anonyma shoal (Sandringham)	-37.9574167	144.98865	5.3	<5-10	North
09 Finger	-38.044183	144.793833	19.4	15-20	Midbay
10 Ricketts natural reef	-38.00345	145.0335	9	<5-10	East
12 Rec reef	-38.036083	145.076967	11.5	10-15	East

13 East Tyre reef	-38.051167	145.0788	12.5	10-15	East
14 East Gaso 4 shallow	-38.046983	145.053667	15	15-20	East
15 Gaso Deep	-38.041283	144.983717	19	15-20	East
16 East Carrum wide	-38.053417	144.94145	20.5	>20	Midbay
17 East Mornington wide	-38.123883	144.940517	22.6	>20	Midbay
18 East Aircraft/Aeroplane	-38.1000167	145.01185	19.4	15-20	East
19 Barge Carrum	-38.07715	145.039033	18	15-20	East
20 East Seaford 16m	-38.09975	145.0564	16	15-20	East
21 Tedesco reef	-38.08735	145.099183	11.5	10-15	East
23 Yakka reef	-38.14135	145.09135	11.5	10-15	East
24 East Woolies natural reef	-38.155	145.090883	8	<5-10	East
25 East Mornington paddock	-38.148083	145.03395	18.8	15-20	East
26 Ansetts	-38.173583	145.034833	20	15-20	East
27 East Mornington hospital	-38.168233	144.9593	21.6	>20	Midbay
28 South Channel #1	-38.331	144.859117	15	15-20	South Channels
30 Symmonds Channel # 1	-38.226833	144.810217	6	<5-10	South Channels
31 South Symmonds Channel #2	-38.23135	144.800517	6	<5-10	South Channels
32 West Channel # 1	-38.205067	144.746	8	<5-10	South Channels
33 South Channel #2	-38.24655	144.718183	7	<5-10	South Channels
35 9ft Bank (Pt Lillias Channel)	-38.101567	144.436933	4	<5-10	Geelong
36 Channel Portarlington	-38.086467	144.628533	11	10-15	Geelong
37 Turning bouy (Clifton Springs)	-38.12525	144.543583	9	<5-10	Geelong
38 West Point Henry	-38.119767	144.42425	5	<5-10	Geelong
39 South The heads 0 (off Queenscliff)	-38.269767	144.67045	5.1	<5-10	Heads

40 South The heads 1	-38.272983	144.673083	15	15-20	Heads
41 South The heads 2	-38.276667	144.676983	15	15-20	Heads
42 South The heads 3	-38.2806	144.679433	15	15-20	Heads
43 South The heads 4	-38.284833	144.682	15	15-20	Heads
44 South The heads 5	-38.28885	144.685517	15.9	15-20	Heads
45 South The heads 6	-38.2941	144.6841	16.1	15-20	Heads
46 South The heads 7	-38.298567	144.681767	15	15-20	Heads
47 South The heads 8 (off Quarantine)	-38.302983	144.680883	16.6	15-20	Heads
56 West Point Wilson Sevengill	-38.060817	144.6923	12.5	10-15	Geelong
57 Midbay 1/ Sevengill Rec 2	-38.160083	144.858633	23	>20	Midbay
58 Midbay 2/ Sevengill Rec 3	-38.226783	144.862067	23	>20	Midbay
59 Midbay 3/ Sevengill Rec 4	-38.075933	144.853783	23	>20	Midbay

Appendix 7.2 Details of acoustic tags

Shark ID	Date Tagged	Area	Tag Type	Tag Family	ID Code	Tag Serial #	Tag ID	Tag life (days)	Power level	Min-Max Delay (sec)	Slope	Intercept	Sex	Total Length (cm)
49	10.04.2015	MidBay	V13	V13-1x	1458	1193997	A69-9001-1458	428	High	80-120			M	73
53	10.04.2015	MidBay	V13	V13-1x	1459	1193998	A69-9001-1459	428	High	80-120			F	77.5
55	10.04.2015	MidBay	V13	V13-1x	1460	1193999	A69-9001-1460	428	High	80-120			F	62
51	10.04.2015	MidBay	V13	V13-1x	1461	1194000	A69-9001-1461	428	High	80-120			M	79
57	10.04.2015	MidBay	V13	V13-1x	1462	1194001	A69-9001-1462	428	High	80-120			M	72
50	10.04.2015	MidBay	V13	V13-1x	1463	1194002	A69-9001-1463	428	High	80-120			M	71.5
54	10.04.2015	MidBay	V13	V13-1x	1464	1194003	A69-9001-1464	428	High	80-120			F	71
52	10.04.2015	MidBay	V13	V13-1x	1465	1194004	A69-9001-1465	428	High	80-120			F	56
56	10.04.2015	MidBay	V13	V13-1x	1466	1194005	A69-9001-1466	428	High	80-120			M	67
15	12.03.2015	Geelong Arm	V13 (depth)	V13P-1x	14726	1127390	A69-9002-14726	879	Low	120-180	0.4397	-1.7587	F	110
44	30.03.2015	Mornington	V13 (depth)	V13P-1x	14730	1127329	A69-9002-14730	879	Low	120-180	0.4397	-1.7587	M	70.5
48	31.03.2015	Geelong Arm	V13 (depth)	V13P-1x	14731	1127330	A69-9002-14731	879	Low	120-180	0.4397	-1.7587	M	73
46	30.03.2015	Mornington	V13 (depth)	V13P-1x	14735	1127334	A69-9002-14735	879	Low	120-180	0.4397	-1.7587	F	63.5
45	30.03.2015	Mornington	V13 (depth)	V13P-1x	14736	1127335	A69-9002-14736	879	Low	120-180	0.4397	-1.7587	M	72
20	29.03.2015	MidBay	V13 (depth)	V13P-1x	14737	1127336	A69-9002-14737	879	Low	120-180	0.4397	-1.7587	F	200
37	30.03.2015	Mornington	V13	V13-1L	31481	1167854	A69-1601-31481	1198	Low	120-180			M	60

42	30.03.2015	Mornington	V13	V13-1L	31482	1167855	A69-1601-31482	1198	Low	120-180	F	70.1
43	30.03.2015	Mornington	V13	V13-1L	31483	1167856	A69-1601-31483	1198	Low	120-180	F	60
47	30.03.2015	Mornington	V13	V13-1L	31484	1167857	A69-1601-31484	1198	Low	120-180	M	61.5
39	30.03.2015	Mornington	V13	V13-1L	31485	1167858	A69-1601-31485	1198	Low	120-180	F	75.5
18	14.03.2015	MidBay	V13	V13-1L	31486	1167859	A69-1601-31486	1198	Low	120-180	F	62
19	25.03.2015	Geelong Arm	V13	V13-1L	31487	1167860	A69-1601-31487	1198	Low	120-180	F	65
25	29.03.2015	MidBay	V13	V13-1L	31488	1167861	A69-1601-31488	1198	Low	120-180	F	70.5
24	29.03.2015	MidBay	V13	V13-1L	31489	1167862	A69-1601-31489	1198	Low	120-180	F	78
23	29.03.2015	MidBay	V13	V13-1L	31490	1167683	A69-1601-31490	1198	Low	120-180	M	74
10	02.03.2015	MidBay	V13	V13-1L	31491	1167864	A69-1601-31491	1198	Low	120-180	F	72
21	29.03.2015	MidBay	V13	V13-1L	31492	1167865	A69-1601-31492	1198	Low	120-180	F	64.5
40	30.03.2015	Mornington	V13	V13-1L	31493	1167866	A69-1601-31493	1198	Low	120-180	M	75.5
31	29.03.2015	MidBay	V13	V13-1L	31494	1167867	A69-1601-31494	1198	Low	120-180	F	64
8	02.03.2015	MidBay	V13	V13-1L	31495	1167868	A69-1601-31495	1198	Low	120-180	F	62
35	30.03.2015	Mornington	V13	V13-1L	31496	1167869	A69-1601-31496	1198	Low	120-180	M	71
36	30.03.2015	Mornington	V13	V13-1L	31497	1167870	A69-1601-31497	1198	Low	120-180	M	70
32	29.03.2015	MidBay	V13	V13-1L	31498	1167871	A69-1601-31498	1198	Low	120-180	F	61.5
41	30.03.2015	Mornington	V13	V13-1L	31499	1167872	A69-1601-31499	1198	Low	120-180	M	71.5
38	30.03.2015	Mornington	V13	V13-1L	31500	1167873	A69-1601-31500	1198	Low	120-180	M	72
9	02.03.2015	MidBay	V13	V13-1L	31501	1167874	A69-1601-31501	1198	Low	120-180	F	60
11	02.03.2015	MidBay	V13	V13-1L	31502	1167875	A69-1601-31502	1198	Low	120-180	F	59
22	29.03.2015	MidBay	V13	V13-1L	31503	1167876	A69-1601-31503	1198	Low	120-180	F	72.5
34	30.03.2015	Mornington	V13	V13-1L	31504	1167877	A69-1601-31504	1198	Low	120-180	M	67
33	29.03.2015	MidBay	V13	V13-1L	31505	1167878	A69-1601-31505	1198	Low	120-180	M	70
29	29.03.2015	MidBay	V13	V13-1L	31506	1167879	A69-1601-31506	1198	Low	120-180	F	60.5
28	29.03.2015	MidBay	V13	V13-1L	31507	1167880	A69-1601-31507	1198	Low	120-180	F	73

27	29.03.2015	MidBay	V13	V13-1L	31508	1167881	A69-1601-31508	1198	Low	120-180	M	67
17	14.03.2015	MidBay	V13	V13-1L	31509	1167882	A69-1601-31509	1198	Low	120-180	M	58
26	29.03.2015	MidBay	V13	V13-1L	31510	1167883	A69-1601-31510	1198	Low	120-180	M	76
90	17.04.2015	South Channel	V16	V16-6x	33127	1150018	A69-1303-33127	2555	Low	120-180	M	217
1	26.11.2014	South Channel	V16	V16-6x	64040	1127446	A69-1601-64040	3650	Low	40-80	F	248
2	26.11.2014	South Channel	V16	V16-6x	64041	1127447	A69-1601-64041	3650	Low	40-80	F	184
4	28.11.2014	Geelong Arm	V16	V16-6x	64042	1127448	A69-1601-64042	3650	Low	40-80	F	240
3	28.11.2014	Geelong Arm	V16	V16-6x	64043	1127449	A69-1601-64043	3650	Low	40-80	F	190
5	28.11.2014	Geelong Arm	V16	V16-6x	64045	1127451	A69-1601-64045	3650	Low	40-80	F	206
30	29.03.2015	MidBay	V16	V16-6x	64046	1127452	A69-1601-64046	3650	Low	40-80	M	210
104	25.04.2015	MidBay	V16	V16-6x	64047	1127453	A69-1601-64047	3650	Low	40-80	M	225
69	10.04.2015	MidBay	V16	V16-6x	64051	1127457	A69-1601-64051	3650	Low	40-80	F	180
92	22.04.2015	MidBay	V16	V16-6x	64053	1127459	A69-1601-64053	3650	Low	40-80	F	215
13	03.03.2015	South Channel	V16	V16-6x	64054	1127460	A69-1601-64054	3650	Low	40-80	F	260
95	22.04.2015	MidBay	V16	V16-6x	64055	1127461	A69-1601-64055	3650	Low	40-80	F	185
94	22.04.2015	MidBay	V16	V16-6x	64056	1127462	A69-1601-64056	3650	Low	40-80	M	213
93	22.04.2015	MidBay	V16	V16-6x	64057	1127463	A69-1601-64057	3650	Low	40-80	F	125
87	12.04.2015	MidBay	V16	V16-6x	64058	1127464	A69-1601-64058	3650	Low	40-80	F	196
91	17.04.2015	South Channel	V16	V16-6x	64059	1127465	A69-1601-64059	3650	Low	40-80	M	218

Appendix 7.3 List of Haplotypes

Hap_1:
CGGATTGTTTTTAGAATCACCGTACATATTTATTTATTAAATATGATACACTAATCGTACACGATTACAAATCGTGCGATTAGTGTATGTCGCGTAAACTATCTTAGAAATTACAC
GTCCTTGGTTAAAACCAAAACCGCACGCATCATGCGGCCCAAAATTCTATACTAGTATAAAAATCATTACATGATTTATGTACGTAAATCATTACATGATTAGTATAAAAATCATT
ACATGATTTATGTACGTAAATCATTACATGATTAACGTATTAATCATTACAATTTTATTAAGAAAAACAAGTCAGTTAGACATCAATTTTATTTGACCCACATTTTACTAAGATAC
GTACATTTCCCACTTTCAATAATCACCTTTAACAGATAATCACCTACTATATAGTTATTTTATACGTAATACTCATCAATTTAAATTCCTCCCTTCAATTACATATTATTATTAAT
CGTGCATATAATTCTAATAAACAATATTTCATTACAATCTATTCTTAATCCTCATAACTGTAATAATCATATTTTGATACCATTAAAAATCTAACTCCTCAATTTCATGATTTCTA
AAATTATTATTGCGGGCTGGTAAGAAATAACCATTACTCTAATACAGGCATATAGTCAACGGTTTGTGGTACGGTTTATCGATAATCCCCTAATATTGATCAAATGCTGGCATTTG
GCTAACTTGAAGTACATACGGTTCAG-ACGCGTCAGAACTCCTAGTCCTCTAGCTCCCTTATATTGACACATGGTTCTTAATCGTCTCATATTGATTGTCCTCCCAGCTTTTTTTT

Hap_2:
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ACATGATTTATGTACGTAAATCATTACATGATTAACGTATTAATCATTACAATTTTATTAAGAAAAACAAGTCAGTTAGACATCAATTTTATTTGACCCACATTTTCCTAAGATAC
GTACATTCCCCACTTTCAATAATCACCTTTAACAGATAATCACCTACTATATAACTATTTTGTACGTAATACTCATTAATTTAAATTCCTCCCTTCAATTACATATTATTATTAAT
CGTGCATATAATTCTAATAAACAATATTTCATTACAACCTATTCTTAATCCTCATAACTGTAATAATCATATTTTGATACCATT-
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CTCATATTGATTGTCCTCCCAGCTTTTTTTT
Hap_3:
CGGATTGTTTTTAGAATCACCGTACATATTTATTTATTAAATATGATACACTAATCGTACACGATTACAAATCGTGCGATTAGTGTATGTCGCGTAAACTATCTTAGAAATTACAC
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CGTGCATATAATTCTAATAAACAATATTTCATTACAACCTATTCTTAATCCTCATAACTGTAACAATCATATTTTGATACCATT-
AAAATCTAACTCCTCAATTTCATGATTTCCAAAATTATTATTGCGGGCTGGTAAGAAATAACCATTACTCTAATACAGGCATATAGTCAACGGTTTGTGGAACGGTTTACCGATAA
TCCCCTAATATTGATCAAATGCTGGCATTTGGCTAACTTGAAGTACATACGGTTCTGTACGCGTCAGAACTCCTAGTCCTCTAGTTCCCTTATATTGACACATGGTTCTTAATCGT

CTCATATTGATTGTCCTCCCAGCTTTTTTTT

Hap_4:
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GTACATTCCCCACTTTCAATAATCACCTTTAACAGATAATCACCTACTATATAACTATTTTGTACGTAATACTCATTAATTTAAATTCCTCCCTTCAATTACATATTATTATTAAT
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CTCATATTGATTGTCCTCCCAGCTTTTTTTT

Hap_5:
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Hap_6:
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Hap_7:

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CGTGCATATAATTCTAATAAACAATATTTCATTACAATCTATTCTTAATCCTCATAACTGTAATAATCATATTTTGATACCATT-
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CTCATATTGATTGTCCTCCCAGCTTTTTTTT

7 References

Abecasis D, Steckenreuter A, Reubens J, Aarestrup K, Alós J, Badalamenti F, Bajona L, Boylan P, Deneudt K, Greenberg L, Brevé N, Hernández F, Humphries N, Meyer C, Sims D, Thorstad EB, Walker AM, Whoriskey F, Afonso P (2018) A review of acoustic telemetry in Europe and the need for a regional aquatic telemetry network. Animal Biotelemetry 6:7

Acuña-Marrero D, Smith ANH, Hammerschlag N, Hearn A, Anderson MJ, Calich H, Pawley MDM, Fischer C, Salinas-de-León P (2017) Residency and movement patterns of an apex predatory shark (*Galeocerdo cuvier*) at the Galapagos Marine Reserve. PLOS ONE 12:e0183669

Akaike H (1981) Likelihood of a Model and Information Criteria. J Econom 16:3–14

Anderson OF, Bagley NW, Hurst RJ, Francis MP, Clark MR, McMillan PJ (1998) Atlas of New Zealand fish and squid distributions from research bottom trawls. NIWA Technical Report 42

Andreotti S, von der Heyden S, Henriques R, Rutzen M, Meÿer M, Oosthuizen H, Matthee CA (2016) New insights into the evolutionary history of white sharks, *Carcharodon carcharias*. Journal of Biogeography 43:328-339

Andrews KS, Williams GD, Farrer D, Tolimieri N, Harvey CJ, Bargmann G, Levin PS (2009) Diel activity patterns of sixgill sharks, *Hexanchus griseus*: the ups and downs of an apex predator. Animal Behaviour 78:525-536

Andrews KS, Williams GD, Levin PS (2010) Seasonal and Ontogenetic Changes in Movement Patterns of Sixgill Sharks. PloS ONE 5:e12549

Ashe JL, Feldheim KA, Fields AT, Reyier EA, Brooks EJ, O'Connell MT, Skomal G, Gruber SH, Chapman DD (2015) Local population structure and context-dependent isolation by distance in a large coastal shark. Marine Ecology Progress Series 520:203-216

Austin D, Bowen WD, McMillan JI (2004) Intraspecific variation in movement patterns: modelling individual behaviour in a large marine predator. Oikos 105:15-30

Avise JC (2000) Phylogeography: The history and formation of species.

Avise JC, Bowen BW, Ayala FJ (2016) In the light of evolution X: Comparative phylogeography. PNAS 113

Awruch CA, Jones SM, Asorey MG, Barnett A (2014) Non-lethal assessment of the reproductive status of broadnose sevengill sharks *Notorynchus cepedianus* to determine the significance of habitat use in coastal areas. Conservation Physiology 2:1-14

Baker CS, Slade RW, Bannister JL, Abernethy RB, Weinrich MT, Lien J, Urban J, Corkeron P, Calmabokidis J, Vasquez O, Palumbi SR (1994) Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. Molecular Ecology 3:313-327

Baker RJ, Bradley RD (2006) Speciation in mammals and the genetic species concept. Journal of mammalogy 87:643-662

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evololution 16:37-48

Barbini SA, Lucifora LO, Figueroa DE (2015) Using opportunistic records from a recreational shing magazine to assess population trends of sharks. . Canadian Journal of Fisheries and Aquatic Sciences 72:1–7

Barnett A, Abrantes K, Stevens JD, Yick JL, Frusher SD, Semmens JM (2010a) Predator-prey relationships and foraging ecology of a marine apex predator with a wide temperate distribution. Marine Ecology Progress Series 416:189-200

Barnett A, Abrantes KG, Stevens JD, Bruce BD, Semmens JM (2010b) Fine-scale movements of the broadnose sevengill shark and its main prey, the gummy shark. PLoS ONE 5:e15464

Barnett A, Abrantes KG, Stevens JD, Semmens JM (2011) Site fidelity and sex-specific migration in a mobile apex predator: implications for conservation and ecosystem dynamics. Animal Behaviour 81:1039-1048

Barnett A, Braccini JM, Awruch CA, Ebert DA (2012) An overview on the role of Hexanchiformes in marine ecosystems: biology, ecology and conservation status of a primitive order of modern sharks. Journal of Fish Biology 80:966-990

Barnett A, Braccini M, Dudgeon CL, Payne NL, Abrantes KG, Sheaves M, Snelling EP (2017) The utility of bioenergetics modelling in quantifying predation rates of marine apex predators: Ecological and fisheries implications. Scientific Reports 7:12982

Barnett A, Semmens JM (2012) Sequential movement into coastal habitats and high spatial overlap of predator and prey suggest high predation pressure in protected areas. Oikos 121:882-890

Barnett A, Stevens JD, Frusher SD, Semmens JM (2010c) Seasonal occurrence and population structure of the broadnose sevengill shark *Notorynchus cepedianus* in coastal habitats of south-east Tasmania. Journal of Fish Biology 77:1688-1701

Beck MW, Heck KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51:633-641

Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L (2015) RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). Molecular Ecology 24:3299-3315

Bernard AM, Feldheim KA, Heithaus MR, Wintner SP, Wetherbee BM, Shivji MS (2016) Global population genetic dynamics of a highly migratory, apex predator shark. Molecular Ecology 25:5312-5329

Bester-van der Merwe AE, Bitalo D, Cuevas JM, Ovenden J, Hernández S, da Silva C, McCord M, Roodt-Wilding R (2017) Population genetics of Southern Hemisphere tope shark (*Galeorhinus galeus*): Intercontinental divergence and constrained gene flow at different geographical scales. PLOS ONE 12:e0184481

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22:148-155

Blackburn JK, Neer JA, Thompson BA (2007) Delineation of bull shark nursery areas in the inland and coastal waters of Louisiana. In: McCandless CT, Kohler NE, Pratt HL (eds) Shark nursery grounds of the Gulf of Mexico and the East Coast waters of the United States American Fishers Society, Bethesda, MD

Block BA, Dewar H, Farwell C, Prince ED (1998) A new satellite technology for tracking the movements of Atlantic bluefin tuna. Proceedings of the National Academy of Science 95:9384–9389

Block BA, Jonsen ID, Jorgensen SJ, Winship AJ, Shaffer SA, Bograd SJ, Hazen EL, Foley DG, Breed GA, Harrison AL, Ganong JE, Swithenbank A, Castleton M, Dewar H, Mate BR, Shillinger GL, Schaefer KM, Benson SR, Weise MJ, Henry RW, Costa DP (2011) Tracking apex marine predator movements in a dynamic ocean. Nature 475:86-90

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120

Bonfil R (1997) Status of shark resources in the southern Gulf of Mexico and Caribbean: implications for management. Fisheries Research 29:101-117

Bonifil R (1994) Overview of world elasmobranch fisheries. FAO Fisheries Technical Paper 341

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A Software Platform for Bayesian Evolutionary Analysis PLoS Comput Biol 10:e1003537

Bowen BW, Clark AM, Abereu-Grobois FA, Chaves A, Reichart HA, Ferl RJ (1998) Global phylogeography of the ridley sea turtles (*Lepidochelys spp.*) as inferred from mitochondrial DNA sequences. Genetica 101:179-189

Bowen BW, Gaither MR, DiBattista JD, Iacchei M, Andrews KR, Grant WS, Toonen RJ, Briggs JC (2016) Comparative phylogeography of the ocean planet. Proceedings of the National Academy of Sciences of the United States of America 113:7962-7969

Braccini JM (2008) Feeding ecology of two high-order predators from south-eastern Australia: the coastal broadnose and the deepwater sharpnose sevengill sharks. Marine Ecology Progress Series 371:273-284

Braccini JM, Rensing K, Langlois T, McAuley R (2017) Acoustic monitoring reveals the broad-scale movement of commercially important sharks. Marine Ecology Progress Series 577:121-129

Bradford RW, Bruce BD, McAuley RB, Robinson G (2011) An evaluation of passive acoustic monitoring using satellite communication technology for near real-time detection of tagged animals in a marine setting. The Open Fish Science Journal 4:10-20

Brewster-Geisz KK, Miller TJ (2000) Management of the sandbar shark, *Carcharhinus plumbeus*: implications of a stage-based model. Fishery Bulletin 98:236-249

Bush A, Holland K (2002) Food limitation in a nursery area: estimates of daily ration in juvenile scalloped hammerheads, Sphyrna lewini (Griffith and Smith, 1834) in Kāne'ohe Bay, Ō'ahu, Hawai'i. Journal of Experimental Marine Biology and Ecology 278:157-178

Calenge C (2006) The package adehabitat for the R software: tool for the analysis of space and habitat use by animals. Ecological Modelling 197:516-519

Camargo SM, Coelho R, Chapman D, Howey-Jordan L, Brooks EJ, Fernando D, Mendes NJ, Hazin FHV, Oliveira C, Santos MN, Foresti F, Mendonça FF (2016) Structure and genetic variability of the oceanic whitetip shark, *Carcharhinus longimanus*, determined using Mitochondrial DNA. PLOS ONE 11:e0155623

Camargo-Gamboa G, Huerta-Quintanilla R, Rodr√≠guez-Achach M (2010) Ecological competition and the role of an apex predator. Physica A: Statistical Mechanics and its Applications 389:4075-4080

Campana SE, Gibson J (2008) Catch and Stock Status of Porbeagle Shark (*Lamna nasus*) in the Northwest Atlantic to 2007. NAFO Doc 08/36

Campana SE, Joyce W, Manning MJ (2009) Bycatch and discard mortality in commercially caught blue sharks *Prionace glauca* assessed using archival satellite pop-up tags. Marine Ecology Progress Series 387:241-253

Campana SE, Marks L, Joyce W, Kohler NE (2006) Effects of recreational and commercial fishing on blue sharks (*Prionace glauca*) in Atlantic Canada, with inferences on the North Atlantic population. Canadian Journal of Fisheries and Aquatic Sciences 63:670–682

Campbell HA, Watts ME, Dwyer RG, Franklin CE (2012) V-Track: software for analysing and visualising animal movement from acoustic telemetry detections. Marine and Freshwater Research 63:815-820

Cardeñosa D, Hyde J, Caballero S (2014) Genetic diversity and population structure of the pelagic thresher shark (*Alopias pelagicus*) in the Pacific Ocean: Evidence for two evolutionarily significant units PloS ONE 9:e110193

Carroll EL, Baker CS, Watson M, Alderman R, Bannister J, Gaggiotti OE, Gröcke DR, Patenaude N, Harcourt R (2015) Cultural traditions across a migratory network shape the genetic structure of southern right whales around Australia and New Zealand. Scientific Reports 5:1-13

Casey JG, Mather FJ, Mason JM, Hoenig J (1978) Offshore fisheries of the Middle Atlantic Bight. Mar Rec Fish 3:107–129

Castro ALF, Stewart BS, Wilson SG, Hueter RE, Meekan MG, Motta PJ, Bowen BW, Karl SA (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). Molecular Ecology 16:5183-5192

Castro JI (1993) The shark nursery of Bulls Bay, South Carolina, with a review of the shark nurseries of the southeastern coast of the United States. Environmental Biology of Fishes 38:37-48

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. Molecular Ecology 22:3124-3140

Cedrola PV, Caille GM, Chaiaramonte GE, Pettovello AD (2009) Demographic structure of broadnose seven-gill shark, *Notorynchus cepedianus*, caught by anglers in southern Patagonia, Argentina. Marine Biodiversity Records 2:1-4

Chabot CL, Espinoza M, Mascareñas-Osorio I, Rocha-Olivares A (2015) The effect of biogeographic and phylogeographic barriers on gene flow in the brown smoothhound shark, *Mustelus henlei*, in the northeastern Pacific. Ecology and Evolution 5:1585-1600

Chapman DD, Feldheim KA, Papastamatiou YP, Heuter RE (2015) There and back again: A review of residency and return migrations in sharks, with implications for population structure and management. Annu Rev Mar Sci 7:547-570

Chen H, Strand M, Norenburg JL, Sun S, Kajihara H, Chernyshev AV, Maslakova SA, Sundberg P (2010) Statistical Parsimony Networks and Species Assemblages in Cephalotrichid Nemerteans (Nemertea). PLOS ONE 5:e12885

Chin A, Heupel MR, Simpfendorfer CA, Tobin AJ (2016) Population organisation in reef sharks: new variations in coastal habitat use by mobile marine predators. Marine Ecology Progress Series 544:197-211

Clarke CR, Karl SA, Horn RL, Bernard AM, Lea JS, Hazin FH, Prodöhl PA, Shivji MS (2015) Global mitochondrial DNA phylogeography and population structure of the silky shark, *Carcharhinus falciformis*. Marine Biology 162:945-955

Clarke SC, Magnussen JE, Abercrombie DL, McAllister M, K.,, Shivji MS (2006a) Identification of Shark Species Composition and Proportion in the Hong Kong Shark Fin Market Based on Molecular Genetics and Trade Records. Conservation Biology 20:201-211

Clarke SC, McAllister M, K.,, Milner-Gulland EJ, Kirkwood GP, Michielsens C, G. J.,, Agnew DJ, Piktch EK, Nakano H, Shivji MS (2006b) Global estimates of shark catches using trade records from commercial markets. Ecological Letters 9:1115-1126

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657-1660

Compagno LJV (1981) Legend versus reality: the jaws image and shark diversity. Oceanus 24:5-16

Compagno LJV (1984) FAO Species Catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1 - Hexanchiformes to Lamniformes., Vol 125. FAO, Rome

Compagno LJV, Dando M, Fowler S (2005) Sharks of the World. Princeton University Press

Cortés E (1999) A stochastic stage-based population model of the sandbar shark in the western north Atlantic. In: Musick JA (ed) Life in the slow lane: ecology and conservation of long lived marine animals, American Fisheries Society Symposium 23, Bethesda, MD

Cosandey-Godin A, Morgan A (2011) Fisheries bycatch of sharks: Options for mitigation. Ocean Science Division, Pew Environment Group, Washington, DC.

Cowman PF, Bellwood DR (2013) Vicariance across major marine biogeographic barriers: temporal concordance and the relative intensity of hard versus soft barriers. Proceedings of the Royal Society B: Biological Sciences 280:20131541

da Silva C, Booth AJ, Dudley SFJ, Kerwath SE, Lamberth SJ, Leslie RW, McCord ME, Sauer WHH, Zweig T (2015) The current status and management of South Africa's chondrichthyan fisheries. African Journal of Marine Science 37:233-248

Daly-Engel TS, Seraphin KD, Holland KN, Coffey JP, Nance HA, Toonen RJ, Bowen BW (2012) Global phylogeography with mixed-marker analysis reveals male-mediated dispersal in the endangered scalloped hammerhead shark (*Sphyrna lewini*). PLOS ONE 7:e29986

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772

De Wysiecki AM, Milessi AC, Wiff R, Jaureguizar AJ (2018) Highest catch of the vulnerable broadnose sevengill shark *Notorynchus cepedianus* in the south-west Atlantic. Journal of Fish Biology 92:543-548

DiBattista JD, Feldheim KA, Thibert-Plante X, Gruber SH, Hendry AP (2008) A genetic assessment of polyandry and breeding-site fidelity in lemon sharks Molecular Ecology 17:3337-3351

Do C, Waples RS, Peel D, MacBeth GM, Tillett BJ, Ovenden JR (2014) NEESTIMATOR v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14:209-214

Domingues RR, Hilsdorf AWS, Shivji MM, Hazin FVH, Gadig OBF (2017) Effects of the Pleistocene on the mitochondrial population genetic structure and demographic history of the silky shark (*Carcharhinus falciformis*) in the western Atlantic Ocean. Rev Fish Biol Fisheries:1-15

Donaldson KA, Wilson RR (1999) Amphi-Panamic Geminates of Snook (Percoidei: Centropomidae) Provide a Calibration of the Divergence Rate in the Mitochondrial DNA Control Region of Fishes. Molecular Phylogenetics and Evolution 13:208-213

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2008) Geneious v4.0.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7:214

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 29:1969–1973

Drymon JM, Ajemian MJ, Powers SP (2014) Distribution and Dynamic Habitat Use of Young Bull Sharks <italic>Carcharhinus leucas</italic> in a Highly Stratified Northern Gulf of Mexico Estuary. PloS ONE 9:e97124

Dudgeon CL, Blower DC, Broderick D, Giles JL, Holmes BJ, Kashiwagi T, Krück NC, Morgan JAT, Tillett BJ, Ovenden JR (2012) A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. Journal of Fish Biology 80:1789-1843

Dudgeon CL, Pollock KH, Braccini JM, Semmens JM, Barnett A (2015) Integrating acoustic telemetry into mark–recapture models to improve the precision of apparent survival and abundance estimates. Oecologia 178:761-772

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LNK, Fordham SV, Francis MP, Pollock CM, Simpfendorfer CA, Burgess GH, Carpenter KE, Compagno LJV, Ebert DA, Gibson C, Heupel MR, Livingstone SR, Sanciangco JC, Stevens JD, Valenti S, White WT (2014) Extinction risk and conservation of the world's sharks and rays. eLife:1-34

Dulvy NK, Simpfendorfer CA, Davidson LNK, Fordham SV, Bräutigam A, Sant G, Welch DJ (2017) Challenges and Priorities in Shark and Ray Conservation. Current Biology 27:R565-R572

Duncan KM, Holland K (2006) Habitat use, growth rates and dispersal patterns of juvenile scalloped hammerhead sharks *Sphyrna lewini* in a nursery habitat. Marine Ecology Progress Series 312:211-221

Duncan KM, Martin AP, Bowen BW, De Couet G (2006) Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). Molecular Ecology 15:2239-2251

Dwyer RG, Brooking C, Brimblecombe W, Campbell HA, Hunter J, Watts M, Franklin CE (2015) An open Web-based system for the analysis and sharing of animal tracking data. Animal Biotelemetry 3:1

Ebert DA (1986) Aspects on the biology of hexanchid sharks along the California coast. In: Uyeno T, Arai R, Tanuchi T, Matsuura M (eds) Second International Conference on Indo-Pacific Fishes, Indo-Pacific Fish Biology Intl Specializatied Book Services Inc., Tokyo

Ebert DA (1989) Life history of the sevengill shark, *Notorynchus cepedianus* Peron, in two northern California bays. California Fish and Game 75:102-112

Ebert DA (1991) Diet of the sevengill shark *N. cepedianus* in the temperate coastal waters of Southern Africa. South African Journal of Marine Science 11:565-572

Ebert DA (1996) Biology of the sevengill shark *Notorynchus cepedianus* (Peron, 1807) in the temperate coastal waters of southern Africa. South African Journal of Marine Science 17:93-103

Ebert DA (2001) Cow Shark. Califonia's Living Marine Resources: A status Report. California Department of Fish and Game

Ebert DA (2002) Ontogenetic changes in the diet of the sevengill shark (*Notorynchus cepedianus*). Marine and Freshwater Research 53:517-523

Ebert DA (2003) Sharks, Rays and Chimaeras of California. University of California Press, Berkeley, CA

Ebert DA, White WT, Ho H-C (2013) Redescription of *Hexanchus nakamurai* Teng 1962, (Chondrichthyes: Hexanchiformes: Hexanchidae), with designation of a neotype. Zootaxa 3752:20-34

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. PloS ONE 6:e19379

Estes JA, Duggins DO (1995) Sea Otters and Kelp Forests in Alaska: Generality and Variation in a Community Ecological Paradigm. Ecological Monographs 65:75-100

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567

Feldheim KA, Gruber SH, DiBattista JD, Babcock EA, Kessel SA, Hendry AP, Pikitch EK, Ashley MV, Chapman DD (2013) Two decades of genetic profiling yields first evidence of natal philopatry and long-term fidelity to parturition sites in sharks. Molecular Ecology:n/a-n/a

Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK (2010) Patterns and ecosystem consequences of shark declines in the ocean. Ecology Letters 13:1055-1071

Frisk MG, Jordaan A, Miller TJ (2014) Moving beyond the current paradigm in marine population connectivity: are adults the missing link? Fish and Fisheries 15:242-254

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

Gaggiotti OE (1996) Population genetic models of source–sink metapopulations. Theoretical Population Biology 50:178-208

Gallucci VF, Taylor IG, Erzini K (2006) Conservation and management of exploited shark populations based on reproductive value. Canadian Journal of Fisheries and Aquatic Sciences 63:931-942

Geraghty PT, Williamson JE, MacBeth WG, Blower DC, Morgan JAT, Johnson G, Ovenden JR, Gillings MR (2014) Genetic structure and diversity of two highly vulnerable carcharhinids in Australian waters. Endangered Species Research 24:45-60

Geraghty PT, Williamson JE, Macbeth WG, Wintner SP, Harry AV, Ovenden JR, Gillings MR (2013) Population expansion and genetic structure in *Carcharhinus brevipinna* in the Southern Indo-Pacific. PLoS ONE 8:e75169

Giles JL, Riginos C, Naylor GJP, Dharmadi, Ovenden JR (2016) Genetic and phenotypic diversity in the wedgefish *Rhynchobatus australiae*, a threatened ray of high value in the shark fin trade. Marine Ecology Progress Series 548:165-180

Gotelli NJ, Hart EM, Ellison AM (2015) EcoSimR: Null model analysis for ecological data. R package version 0.1.0.

Guttridge TL, Gruber SH, Franks BR, Kessel ST, Gledhill KS, Uphill J, Krause J, Sims DW (2012) Deep danger: intra-specific predation risk influences habitat use and aggregation formation of juvenile lemon sharks *Negaprion brevirostris*. Marine Ecology Progress Series 445:279-291

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A Global Map of Human Impact on Marine Ecosystems. Science 319:948-952

Hamer P, Conron S (2016) Snapper Stock Assessment. Fisheries Victoria, Fisheries Management and Science Branch, Queenscliff

Hamer P, Mills K (2017) Anglers tune in to find out why Port Phillip Bay is so important to snapper. Recreational Fishing Grants Program Research Report. Victorian Government, Department of Economic Development, Jobs, Transport and Resources

Hamer PA, Acevedo S, Jenkins GP, Newman A (2011) Connectivity of a large embayment and coastal fishery: spawning aggregations in one bay source local and broad-scale fishery replenishment. Journal of Fish Biology 78:1090-1109

Harpending RC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum Biol 66

Harris G, Batley G, Fox D, Hall D, Jernakoff P, Molloy R, Murray A, Newell B, Parslow J, Skyring G, Walker S (1996) Port Phillip Bay Environmental Study. Final Report, CSIRO, Canberra, Australia

Harrison S, Hastings A (1996) Genetic and evolutionary consequences of metapopulation structure. Trends in Ecology & Evolution 11:180-183

Hart MW, Sunday J (2007) Things fall apart: biological species form unconnected parsimony networks. Biology Letters 3:509-512

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270:313–321

Heist EJ, Musick JA, Graves JE (1996) Genetic population structure of the shortfin mako (*Isurus oxyrinchus*) inferred from restriction fragment length polymorphism analysis of mitochondrial DNA. Canadian Journal of Fisheries and Aquatic Sciences 53:583-588

Heithaus M, Dill L, Marshall G, Buhleier B (2002) Habitat use and foraging behavior of tiger sharks (*Galeocerdo cuvier*) in a seagrass ecosystem. Marine Biology 140:237-248

Heithaus MR (2007) Nursery areas as essential shark habitats: a theoretical perspective American Fisheries Society Symposium 50:3–13

Heithaus MR, Frid A, Wirsing AJ, Worm B (2008) Predicting ecological consequences of marine top predator declines. Trends in Ecology & Evolution 23:202-210

Heppell SS, Crowder L.B., T.R. M (1999) Life table analysis of long-lived marine species with implications for conservation and management. In: Musick JA (ed) Life in the slow lane: ecology and conservation of long lived marine animals, American Fisheries Society Symposium 23, Bethesda, MD

Heupel MR, Carlson JK, Simpfendorfer CA (2007) Shark nursery areas: concepts, definition, characterization and assumptions. Marine Ecology Progress Series 337:287-297

Heupel MR, Hueter RE (2002) Importance of prey density in relation to the movement patterns of juvenile blacktip sharks (*Carcharhinus limbatus*) within a coastal nursery area. Marine and Freshwater Research 53:543-550

Heupel MR, Kanno S, Martins APB, Simpfendorfer CA (2018) Advances in understanding the roles and benefits of nursery areas for elasmobranch populations. Marine and Freshwater Research:-

Heupel MR, Semmens JM, Hobday AJ (2006) Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. Marine and Freshwater Research 57:1-13

Heupel MR, Simpfendorfer CA (2002) Estimation of mortality of juvenile blacktip sharks, *Carcharhinus limbatus*, within a nursery area using telemetry data. Canadian Journal of Fisheries and Aquatic Sciences 59:624-632

Heupel MR, Simpfendorfer CA (2011) Estuarine nursery areas provide a low-mortality environment for young bull sharks *Carcharhinus leucas*. Marine Ecology Progress Series 433:237-244

Heupel MR, Simpfendorfer CA, Hueter RE (2004) Estimation of shark home ranges using passive monitoring techniques. Environmental Biology of Fishes 71:135-142

Heupel MR, Webber DM (2012) Trends in acoustic tracking: Where are the fish going and how will we follow them? American Fisheries Society Symposium 76:219-231

Heupel MR, Yeiser BG, Collins AB, Ortega L, Simpfendorfer CA (2010) Long-term presence and movement patterns of juvenile bull sharks, *Carcharhinus leucas*, in an estuarine river system. Marine and Freshwater Research 61:1-10

Hoelzel AR, Shivji MS, Magnussen J, Francis MP (2006) Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). Biology Letters 2:639-642

Holland KN, Lowe CG, Peterson JD, Gill A (1992) Tracking coastal sharks with small boats: Hammerhead shark pups as a case study. Australian Journal of Marine and Freshwater Research 43:61-66

Holmes BJ, Williams SM, Otway NM, Nielsen EE, Maher SL, Bennett MB, Ovenden JR (2017) Population structure and connectivity of tiger sharks (*Galeocerdo cuvier*) across the Indo-Pacific Ocean basin. Royal Society Open Science 4:170309

Hoyos-Padilla EM, Klimley AP, Galván-Magaña F, Antoniou A (2016) Contrasts in the movements and habitat use of juvenile and adult white sharks (*Carcharodon carcharias*) at Guadalupe Island, Mexico. Animal Biotelemetry 4:14

Hueter RE, Heupel MR, Heist EJ, Keeney DB (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. Journal of Northwest Atlantic Fishery Science 35:239-247

Hussey NE, McCarthy ID, Dudley SFJ, Mann BQ (2009) Nursery grounds, movement patterns and growth rates of dusky sharks, *Carcharhinus obscurus*: a long-term tag and release study in South African waters. Marine and Freshwater Research 60:571-583

Iacchei M, Ben-Horin T, Selkoe KA, Bird CE, García-Rodríguez FJ, Toonen RJ (2013) Combined analyses of kinship and F(ST) suggest potential drivers of chaotic genetic patchiness in high gene-flow populations. Molecular Ecology 22:3476-3494

IMOS (2018) Animal Tracking Database. http://animaltracking.aodn.org.au, accessed 2018

Irigoyen A, Trobbiani G (2016) Depletion of trophy large-sized sharks populations of the Argentinean coast, south-western Atlantic: insights from fishers' knowledge Neotropical Ichthyology 14:e150081

IUCN (2012) IUCN Red List of Threatened Species. Version 2012.2. Accessed on 24 February 2013.

Jackson EL, Rowden AA, Attrill MJ, Bossey SJ, Jones MB (2001) The importance of seagrass beds as a habitat for fishery species. Oceanography and Marine Biology 39:269-303

Jeltsch F, Bonte D, Pe'er G, Reineking B, Leimgruber P, Balkenhol N, Schröder B, Buchmann CM, Mueller T, Blaum N, Zurell D, Böhning-Gaese K, Wiegand T, Eccard JA, Hofer H, Reeg J, Eggers U, Bauer S (2013) Integrating movement ecology with biodiversity research - exploring new avenues to address spatiotemporal biodiversity dynamics. Movement Ecology 1:6

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94

Jones CM (2006) Estuarine and diadromous fish populations. In: Kritzer JP, Sale PF (eds) Marine Metapopulations. Academic Press, San Diego, CA

Jorgensen SJ, Reeb CA, Chapple TK, Anderson S, Perle C, Van Sommeran SR, Fritz-Cope C, Brown AC, Klimley AP, Block BA (2009) Philopatry and migration of Pacific white sharks. Proceegins of the Royal Society B 277:678-688

Karl SA, Castro ALF, Lopez JA, Charvet P, Burgess GH (2011) Phylogeography and conservation of the bull shark (*Carcharhinus leucas*) inferred from mitochondrial and microsatellite DNA. Conservation Genetics 12:371-382

Keeney DB, Heist EJ (2006) Worldwide phylogeography of the blacktip shark (*Carcharhinus limbatus*) inferred from mitochondrial DNA reveals isolation of western Atlantic populations coupled with recent Pacific dispersal. Molecular Ecology 15:3669-3679

Keeney DB, Heupel MR, Hueter RE, Heist EJ (2005) Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea Molecular Ecology 14:1911-1923

Ketchum JT, Hearn A, Klimley AP, Espinoza E, Peñaherrera C, Largier JL (2014) Seasonal changes in movements and habitat preferences of the scalloped hammerhead shark (*Sphyrna lewini*) while refuging near an oceanic island. Marine Biology:1-13

Ketchum JT, Slager CJ, Buckhorn ML, Nosal AP, Peter Klimley A (2017) Residency and long-distance movements of sevengill sharks (Notorhynchus cepedianus) tagged in San Francisco Bay. Animal Biotelemetry 5:26

Kinney MJ, Simpfendorfer CA (2009) Reassessing the value of nursery areas to shark conservation and management. Conservation Letters 2:53-60

Knip DM, Heupel MR, Simpfendorfer CA (2010) Sharks in nearshore environments: models, importance, and consequences. Marine Ecology Progress Series 402:1-11

Kotta J, Wernberg T, Jänes H, Kotta I, Nurkse K, Pärnoja M, Orav-Kotta H (2018) Novel crab predator causes marine ecosystem regime shift. Scientific Reports 8:4956

Kumar R, Kumar V (2018) A review of phylogeography: biotic and abiotic factors. Geology, Ecology, and Landscapes:1-7

Labrado P, Rozas J (2009) DnaSP v5: A software for comprhensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452

Lah L, Trense D, Benke H, Berggren P, Gunnlaugsson Þ, Lockyer C, Öztürk A, Öztürk B, Pawliczka I, Roos A, Siebert U, Skóra K, Víkingsson G, Tiedemann R (2016) Spatially explicit analysis of genome-wide SNPs detects subtle population structure in a mobile marine mammal, the harbor porpoise. PLOS ONE 11:e0162792

Larson S, Farrer D, Lowry D, Ebert DA (2015) Preliminary Observations of Population Genetics and Relatedness of the Broadnose Sevengill Shark, *Notorynchus cepedianus*, in Two Northeast Pacific Estuaries. PloS ONE 10:1-11

Larson SE, Daly-Engel TS, Phillips NM (2017) Chapter Three - Review of Current Conservation Genetic Analyses of Northeast Pacific Sharks. In: Larson SE, Lowry D (eds) Advances in Marine Biology, Book 77. Academic Press

Larson WA, Seeb LW, Everett MV, Waples RK, Templin WD, Seeb JE (2014) Genotyping by sequencing resolves shallow population structure to inform conservation of Chinook salmon (*Oncorhynchus tshawytscha*). Evolutionary Applications 7:355-369

Last PR, Stevens JD (2009) Sharks and Rays of Australia. 2nd edn. CSIRO Publishing, Melbourne

Lee RS, Black KP, Bosserelle C, Greer D (2012) Present and future prolonged drought impacts on a temperate marine embayment. Ocean Dynamics 62:907-922

Lessios HA, Kessing BD, Robertson DR (1998) Massive gene flow across the world's most potent marine biogeographic barrier. Proceedings of the Royal Society B: Biological Sciences 265:583-588

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic-structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). American Journal of Botany 82:1420-1425

Lucifora LO, García VB, Worm B (2011) Global diversity hotspots and conservation priorities for sharks. PLOS ONE 6:e19356

Lucifora LO, Menni RC, Escalante AH (2005) Reproduction, abundance and feeding habits of the broadnose sevengill shark *Notorynchus cepedianus* in north Patagonia, Argentina. Marine Ecology Progress Series 289:237-244

Lynch AMJ, Sutton SG, Simpfendorfer CA (2010) Implications of recreational fishing for elasmobranch conservation in the Great Barrier Reef Marine Park. Aquatic Conservation-Marine and Freshwater Ecosystems 20:312-318

Maduna SN, Rossouw C, da Silva C, Soekoe M, Bester-van der Merwe A (2017) Species identification and comparative population genetics of four coastal houndsharks based on novel NGS-mined microsatellites. Ecology and Evolution:1-25

Maisey JG (2012) What is an 'elasmobranch'? The impact of palaeontology in understanding elasmobranch phylogeny and evolution. Journal of Fish Biology 80:918-951

Manly BFJ, McDonald LL, Thomas DL, McDonald TL, Erickson WP (2003) Resource selection by animals. Statistical design and analysis for field studies, 2nd edition Kluwer Academic Punlishers, Dordrecht, The Netherlands

Martin AP, Naylor GJP, Palumbi SR (1992) Rates of Mitochondrial DNA evolution in sharks are slow compared with mammals. Nature 357:153-155

Martin TG, Chadès I, Arcese P, Marra PP, Possingham HP, Norris DR (2007) Optimal Conservation of Migratory Species. PLoS ONE 2:e751

Martinez-Takeshita N, Purcell CM, Chabot CL, Craig MT, Paterson CN, Hyde JR, Allen LG (2015) A tale of three tails: Cryptic speciation in a globally distributed marine fish of the Genus *Seriola*. Copeia 103:357-368

Matich P, Heithaus MR (2012) Effects of an extreme temperature event on the behavior and age structure of an estuarine top predator, Carcharhinus leucas. Marine Ecology Progress Series 447:165-178

McAllister JD, Barnett A, Lyle JM, Semmens JM (2015) Examining the functional role of current area closures used for the conservation of an overexploited and highly mobile fishery species. ICES Journal of Marine Science:1-11

McAllister JD, Barnett A, Lyle JM, Stehfest KM, Semmens JM (2018) Examining trends in abundance of an overexploited elasmobranch species in a nursery area closure. Marine and Freshwater Research 69:376-384

McFarlane G, King J, Leask K, Christensen LB (2009) Assessment of information used to develop a Recovery Potential Assessment for basking shark *Cetorhinus maximus* (Pacific Population) in Canada. DFO Canadian Science Advisory Secretariat Research Document. 2008/071

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Molecular Ecology Notes 4:792-794

Merson RR, Pratt HLJ (2001) Distribution, Movements and Growth of Young Sandbar Sharks, *Carcharhinus Plumbeus*, in the Nursery Grounds of Delaware Bay. Environmental Biology of Fishes 61:13-24

Meyer CG, Clark TB, Papastamatiou YP, Whitney NM, Holland KN (2009) Long-term movement patterns of tiger sharks *Galeocerdo cuvier* in Hawaii. Marine Ecology Progress Series 381:223-235

Molina J, Cooke S (2012) Trends in shark bycatch research: current status and research needs. Reviews in Fish Biology and Fisheries 22:719-737

Morin PA, Martien KK, Taylor BL (2009) Assessing statistical power of SNPs for population structure and conservation studies. Molecular Ecology Resources 9:66-73

Morrissey JF, Gruber SH (1993) Habitat selection by juvenile lemon sharks, *Negaprion brevirostris*. Environmental Biology of Fishes 38:311-319

Moura AE, Natoli A, Rogan E, Hoelzel AR (2013) Atypical panmixia in a European dolphin species (*Delphinus delphis*): implications for the evolution of diversity across oceanic boundaries. Journal of Evolutionary Biology 26:63-75

Musick JA (1999) Ecology and conservation of long-lived marine animals. In: Musick JA (ed) Life in the slow lane: Ecology and conservation of long-lived marine animals. American Fisheries Society Symposium 23, Bethesda, MD, USA

Musick JA (2005) Management of sharks and their relatives (Elasmobranchii). In: Musick JA, Bonifil R (eds) Elasmobranch fisheries management techniques. FAO, FAO Fisheries Technical Paper. No. 474. Rome

Musick JA, Bonfil R (2005) Management techniques for elasmobranch fisheries. FAO Fisheries Technical Paper No 474. FAO, Rome

Musick JA, Musick S (2011) Sharks. FAO Fisheries and Aquaculture Reviews and Studies. FAO, Rome

Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading Effects of the Loss of Apex Predatory Sharks from a Coastal Ocean. Science 315:1846-1850

Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA (2013) Genotyping-by-sequencing in ecological and conservation genomics. Molecular Ecology 22:2841-2847

Naylor GJP, Caira JN, Jensen K, Rosana KAM, White WT, Last PR (2012) A DNA Sequence–Based approach to the identification of Shark and Ray species and its implications for global Elasmobranch diversity and parasitology. Bulletin of the American Museum of Natural History:262pp

O'Leary SJ, Feldheim KA, Fields AT, Natanson LJ, Wintner S, Hussey N, Shivji MS, Chapman DD (2015) Genetic Diversity of White Sharks, *Carcharodon carcharias*, in the Northwest Atlantic and Southern Africa. Journal of Heredity 106:258-265

Oh BZL, Thums M, Babcock RC, Meeuwig JJ, Pillans RD, Speed C, Meekan MG (2017) Contrasting patterns of residency and space use of coastal sharks within a communal shark nursery. Marine and Freshwater Research:1-17

Olsen A (1954) The Biology, Migration, and Growth Rate of the School Shark, *Galeorhinus australis* (Macleay) (Carcharhanidae) in the South-eastern Australian Waters. Marine and Freshwater Research 5:353-410

Palsbøll PJ, Berube M, Allendorf FW (2006) Identification of management units using population genetic data. TRENDS in Ecology and Evolution 22:11-16

Palumbi SR (1994) Genetic Divergence, Reproductive Isolation, and Marine Speciation. Annual Review of Ecology and Systematics 25:547-572

Pazmiño DA, Maes GE, Simpfendorfer CA, Salinas-de-León P, van Herwerden L (2017) Genome-wide SNPs reveal low effective population size within confined management units of the highly vagile Galapagos shark (*Carcharhinus galapagensis*). Conservation Genetics:1-13

Péron F (1807a) *Notorynchus cepedianus*: In: Database of modern sharks, rays and chimaeras, www.shark-references.com, World Wide Web electronic publication, Version 05/2017 http://shark-references.com/species/view/Notorynchus-cepedianus

Péron F (1807b) Voyage de Découvertes aux Terres Australes, exécuté par ordre de sa majesté l'Empereur et Roi, sur les Corvettes la Géographe, la Naturaliste et la Goulette le Casuarina, pendant les années 1800, 1801, 1803 et 1804. Paris. Voyage de Découvertes aux Terres Australes 1:1-496

Peterson CD, Belcher CN, Bethea DM, Driggers WB, Frazier BS, Latour RJ (2017) Preliminary recovery of coastal sharks in the south-east United States. Fish and Fisheries 18:845-859

Pinsky ML, Palumbi SR (2014) Meta-analysis reveals lower genetic diversity in overfished populations. Molecular Ecology 23:29-39

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-Based Species Delimitation for the DNA Taxonomy of Undescribed Insects. Systematic Biology 55:595-609

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Prugh LR, Stoner CJ, Epps CW, Bean WT, Ripple WJ, Laliberte AS, Brashares JS (2009) The Rise of the Mesopredator. BioScience 59:779-791

Queiroz N, Humphries NE, Mucientes G, Hammerschlag N, Lima FP, Scales KL, Miller PI, Sousa LL, Seabra R, Sims DW (2016) Ocean-wide tracking of pelagic sharks reveals extent of overlap with longline fishing hotspots. PNAS 113:1582 - 1587

Raj A, Stephens M, Pritchard JK (2014) fastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets Genetics 197:573-589 [Genetics, Biorxiv]

Rechisky EL, Wetherbee BM (2003) Short-term movements of juvenile and neonate sandbar sharks, *Carcharhinus plumbeus*, on their nursery grounds in Delaware Bay. Environmental Biology of Fishes 68:113-128

Reiss H, Hoarau G, Dickey-Collas M, Wolff WJ (2009) Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish and Fisheries 10:361-395

Ripple WJ, Beschta RL (2012) Trophic cascades in Yellowstone: The first 15 years after wolf reintroduction. Biological Conservation 145:205-213

Ripple WJ, Larsen EJ, Renkin RA, Smith DW (2001) Trophic cascades among wolves, elk and aspen on Yellowstone National Park,Äôs northern range. Biological Conservation 102:227-234

Ritchie EG, Elmhagen B, Glen AS, Letnic M, Ludwig G, McDonald RA (2012) Ecosystem restoration with teeth: what role for predators? Trends in Ecology and Evolution 27:265-271

Robinson RA, Crick HQP, Learmonth JA, Maclean IMD, Thomas CD, Bairlein F, Forchhammer MC, Francis CM, Gill JA, Godley BJ, Harwood J, Hays GC, Huntley B, Hutson AM, Pierce GJ, Rehfisch MM, Sims DW, Santos BM, Sparks TH, Stroud DA, Visser ME (2009) Travelling through a warming world: climate change and migratory species. Endangered Species Research 7:87-99

Rubio-Castro GG, Quiñonez-Velázquez, García-Rodríguez FJ (2016) Genetic diversity and population structure of Indo-Pacific sailfish *Istiophorus platypterus* in the eastern Pacific. Fish Sci 82:241-249

Rus Hoelzel A, Mahmood SS, Magnussen J, Francis MP (2006) Low worldwide genetic diversity in basking shark (*Cetorhinus maximus*). Biology Letters 2:639-642

Sandoval Laurrabaquio-A N, Islas-Villanueva V, Adams DH, Uribe-Alcocer M, Alvarado-Bremer JR, Díaz-Jaimes P (2019) Genetic evidence for regional philopatry of the Bull Shark (*Carcharhinus leucas*), to nursery areas in estuaries of the Gulf of Mexico and western North Atlantic ocean. Fisheries Research 209:67-74

Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA, Webster MS (2010) Population diversity and the portfolio effect in an exploited species. Nature 465:609–612

Schmidt JV, Schmidt CL, Ozer F, Ernst RE, Feldheim KA, Ashley MV, Levine M (2009) Low genetic differentiation across three major ocean populations of the Whale shark, *Rhincodon typus*. PLoS ONE 4:4988-4988

Schmidt-Roach S, Miller KJ, Lundgren P, Andreakis N (2014) With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. Zoological Journal of the Linnean Society 170:1-33

Schultz JK, Feldheim KA, Gruber SH, Ashley MV, McGovern TM, Bowen BW (2008) Global phylogeography and seascape genetics of the lemon shark (genus *Negaprion*). Molecular Ecology 17:5336-5348

Shivji MS, Clarke S, Pank M, Natanson LJ, Kohler N, Stanhope M (2002) Genetic identification of pelagic shark body parts for conservation and trade monitoring. Conservation Biology 16:1035-1039

Silliman BR, Angelini C (2012) Trophic Cascades Across Diverse Plant Ecosystems. Nature Education Knowledge 3:44

Simpfendorfer CA (1999) Demographic analysis of the dusky shark fishery in southwestern Australia. In: Musick JA (ed) Life in the slow lane: ecology and conservation of long lived marine animals, American Fisheries Society Symposium 23, Bethesda, MD

Simpfendorfer CA (2014) Sharks and rays threatened worldwide - overfishing to blame. Book 2014, The Conversation, Environment + Energy. http://theconversation.com/sharks-and-rays-threatened-worldwide-overfishing-to-blame-22186

Simpfendorfer CA, Dulvy NK (2017) Bright spots of sustainable shark fishing. Current Biology 27:R97–R98

Simpfendorfer CA, Kyne PM (2009) Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. Environmental Conservation 36:97-103

Simpfendorfer CA, Milward NE (1993) Utilisation of a tropical bay as a nursery area by sharks of the families Carcharhinidae and Sphyrnidae. Environmental Biology of Fishes 37:337-345

Sims DW (2003) Tractable models for testing theories about natural strategies: foraging behaviour and habitat selection of free-ranging sharks. Journal of Fish Biology 63:53-73

Sims DW, Southall EJ, Quayle VA, Fox AM (2000) Annual social behaviour of basking sharks associated with coastal front areas. Proc R Soc Lond B 267:1897-1904

Skomal GB (2007) Evaluating the physiological and physical consequences of capture on post-release survivorship in large pelagic fishes. Fisheries Management and Ecology 14:81-89

Skomal GB, Braun CD, Chrisholm JH, Thorrold SR (2017) Movements of the white shark *Carcharodon carcharias* in the North Atlantic Ocean. Marine Ecology Progress Series 580:1-16

Smith SE, Au DW, Show C (1999) Intrinsic rebound potentials of 26 species of Pacific sharks. Marine and Freshwater Research 49:663-678

Spaet JLY, Jabado RW, Henderson AC, Moore ABM, Berumen ML (2015) Population genetics of four heavily exploited shark species around the Arabian Peninsula. Ecology and Evolution 5:2317-2332

Speed CW, Field IC, Meekan MG, Bradshaw CJA (2010) Complexities of coastal shark movements and their implications for management. Marine Ecology Progress Series 408:275-293

Städele V, Vigilant L (2016) Strategies for determining kinship in wild populations using genetic data. Ecology and Evolution 6:6107-6120

Stehfest KM, Patterson TA, Barnett A, Semmens JM (2014) Intraspecific differences in movement, dive behavior and vertical habitat preferences of a key marine apex predator. Marine Ecology Progress Series 495:249-262

Stehfest KM, Patterson TA, Barnett A, Semmens JM (2015) Markov models and network analysis reveal sex-specific differences in the space-use of a coastal apex predator. Oikos 124:307-318

Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES Journal of Marine Science: Journal du Conseil 57:476-494

Stevens JD, West GJ (1997) Investigation of School and Gummy Shark Nursery Areas in South Eastern Australia. Fisheries Research and Development Corporation Project 93/061 Final Report. CSIRO Marine Research., Hobart

Suchanek TH (1994) Temperate Coastal Marine Communities: Biodiversity and Threats. American Zoologist 34:100-114

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochon- drial DNA in humans and chimpanzees. Molecular biology and evolution 10:512-526

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30:2725-2729

Tanaka K, Shiina T, Tomita T, Suzuki S, Hosomichi K, Sano K, Doi H, Kono A, Komiyama T, Inoko H, Kulski JK, Tanaka S (2013) Evolutionary relations of hexanchiformes deep-sea sharks elucidated by whole mitochondrial genome sequences. BioMed Research International:1-11

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. . Lectures Math Life Sci 17:57–86

Tavares R, Rodriguez JP, Morales M (2016) Nursery area and size structure of the lemon shark population, *Negaprion brevirostris* (Poey, 1868), in Los Roques Archipelago National Park, Venezuela. Univ Sci 21:33-52

Thompson KF, Patel S, Baker CS, Constantine R, Millar CD (2016) Bucking the trend: genetic analysis reveals high diversity, large population size and low differentiation in a deep ocean cetacean. Heredity 116:277-285

Veríssimo A, Sampaio í, McDowell JR, Alexandrino P, Mucientes G, Queiroz N, da Silva C, Jones CS, Noble LR (2017) World without borders-genetic population structure of a highly migratory marine predator, the blue shark (*Prionace glauca*). Ecology and Evolution:1-14

Walker TI (1998) Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. Marine and Freshwater Research 49:553-572

Walker TI (1999) Galeorhinus galeus fisheries of the world. In: Shotton R (ed) Case Studies of the Management of Elasmobranch Fisheries FAO Fisheries Technical Paper No 378/2. FAO, Rome, Italy

Walker TI, Hudson RJ, Gason AS (2005) Catch Evaluation of Target, By-product and By-catch Species Taken by Gillnets and Longlines in the Shark Fishery of South- eastern Australia. Journal of Northwest Atlantic Fishery Science 35:505-530

Wallace SS, McFarlane GA, Campana SE, King JR (2009) Status of spiny dogfish in Atlantic and Pacific Canada. In: Gallucci VF, McFarlane GA, Bargmann GG (eds) Biology and management of dogfish sharks. American Fisheries Society, Bethesda, Maryland

Wallach AD, Izhaki I, Toms JD, Ripple WJ, Shanas U (2015) What is an apex predator? Oikos 124:1453-1461

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population Structure. Evolution 38:1358-1370

Wheeler QD (1999) Why the Phylogenetic Species Concept?—Elementary. Journal of Nematology 31:134-141

Williams G, Andrews K, Farrer D, Bargmann G, Levin P (2011) Occurrence and biological characteristics of broadnose sevengill sharks (*Notorynchus cepedianus*) in Pacific Northwest coastal estuaries. Environmental Biology of Fishes 91:379-388

Williams GD, Andrews KS, Katz SL, Moser ML, Tolimieri N, Farrer DA, Levin PS (2012) Scale and pattern of broadnose sevengill shark *Notorynchus cepedianus* movement in estuarine embayments. Journal of Fish Biology 80:1380-1400

Worm B, Davis B, Kettemer L, Ward-Paige CA, Chapman D, Heithaus MR, Kessel ST, Gruber SH (2013) Global catches, exploitation rates, and rebuilding options for sharks. Marine Policy 40:194-204

Zhou S, Smith A, Fuller M (2007) Rapid quantitative risk assessment for fish species in major Commonwealth fisheries. Report to the Australian Fisheries Management Authority, Canberra

Zhou S, Smith A, Fuller M (2009) Rapid quantitative risk assessment for fish species in seven Commonwealth fisheries. Report to the Australian Fisheries Management Authority, Canberra